Saskatchewan
Agriculture, Food
and Rural
Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

19990354

EVALUATION OF NATURALLY OCCURRING MICROORGANISMS FOR BIOLOGICAL CONTROL OF SCENTLESS CHAMOMILE

Funded by: The Agriculture Development Fund

July, 2002
Prepared by: Ag Canada-Receiver General for Canada

105-05130/

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Saskatchewan Agriculture and Food

Research Branch

Agriculture Development Fund

FINAL REPORT
ADF Project 19990354

June, 2002

AGRICULTURE DEVELOPMENT FUND
JUN 2002
REGINA, SASK.
S4S 0B1
PROVINCE of SASKATCHEWAN

Evaluation of Naturally Occurring Microorganisms for Biological Control of Scentless Chamomile

Gary Peng and Karen Bailey

Saskatoon Research Centre, Agriculture and Agri-Food Canada
107 Science Place, Saskatoon, SK S7N 0X2

Abstract

A research project was initiated to evaluate naturally occurring indigenous microorganisms for potential biological control of scentless chamomile in western Canada. Extensive field surveys were carried out during the growing season of 2000 and 2001 to collect disease-causing agents. A total of 505 microbial isolates were obtained from 53 rural municipalities (RMs) across the province, and 218 pathogenic isolates were applied as foliar sprays for weed control. Twenty-seven of the isolates that caused either severe disease or dramatic fresh weight reduction of scentless chamomile were selected for further studies. Several isolates of a pathogen resulted in high mortality of scentless chamomile when applied at high doses, but had no negative impact on crops including cereals, oilseeds, pulses, and forages. When compared to similar organisms obtained from Europe, the indigenous pathogen was equally effective in controlling scentless chamomile, reducing plant fresh weight by up to 70% under a controlled environment. At 20° C, isolates of this indigenous pathogen required a dew period of up to 24 h to cause maximal damage to scentless chamomile, therefore formulation would likely be necessary when used in the field. Our study also showed that these isolates could be applied using common spray equipment at a practical volume such as 200 L/ha through adjusting the spray droplet size or spore concentration. When applied following the herbicide Basagran or Sencor, this indigenous pathogen dramatically enhanced the level of weed control in comparison to either fungus or herbicide alone. There was clearly a synergy between the herbicides and biocontrol fungus. The research showed that there is potential for biological control of scentless chamomile. Further studies should be pursued to capture the value of this project and develop the organism with the most potential.

Executive Summary

Scentless chamomile is a noxious weed in Saskatchewan. Recent surveys showed that infestation was widespread in the province, especially in dark brown and black-soil areas where productions of some high-value specialty crops are concentrated. Scentless chamomile is a difficult weed to control due to its competitiveness and tolerance to most post-emergent herbicides. Conventional measures including tillage are often inadequate. Crop losses can be substantial in cereals, pulses and forages if the weed is not kept under control. It is apparent that new strategies are needed for effective control of scentless chamomile in Saskatchewan.

Biocontrol using plant pathogens represents a potential new tool in integrated weed management. However, information is quite lacking on scentless chamomile in terms of the potential of using this strategy. This project is to identify indigenous pathogens on scentless chamomile through extensive field surveys and to evaluate their potential as biocontrol agents. A total of 505 microbial isolates were collected from 53 RMs in the province where infestation of scentless chamomile had been previously reported. The majority of the isolates collected were fungal organisms. A bioassay was developed to rapidly determine the pathogenicity of these organisms. Pathogenic isolates were further evaluated for weed control on whole plants of scentless chamomile.

A total of 263 fungal isolates were identified as pathogenic with the bioassay, and 218 of them were applied individually with foliar spray to scentless chamomile to test for their ability of weed control. These isolates showed a range of weed control, from highly effective to completely ineffective. Several isolates resulted in weed mortality in the greenhouse, which had not been seen with any chamomile pathogens collected previously. Twenty-seven isolates that caused significant damage to scentless chamomile (> 50% control) were retained for further studies.

A two-tiered system, progressively using more plant species, was used to determine the safety of the 27 fungal isolates on crops commonly grown on the prairies. Initial host-range testing employed 5 crop species; wheat, canola (*Brassica rapa*), lentil, chickpea and flax, to eliminate potential crop pathogens. This test revealed that three isolates, all identified as *Fusarium sporotrichioides*, caused significant disease on canola, lentil, and flax. Several other isolates, primarily *Bipolaris* spp. and *Alternaria* spp., caused noticeable disease on wheat, canola, lentil, or flax and were considered as potential crop pathogens as well. However, thirteen isolates (species identity unknown), resulted in

severe damage only to scentless chamomile. This initial test quickly eliminated detrimental crop pathogens from further studies.

Seven of the thirteen isolates were applied to 14 plant species including most field crops and several closely related native plant species. The majority of these isolates were very host specific, causing disease only on scentless chamomile and a closely related species, *Matricaria recutita* (German chamomile). Considering the high dose of inoculum applied and extended dew period provided after inoculation, the testing conditions were considered optimal for pathogen infection and the risk of disease escape should be low. Therefore the results are a strong indication that these isolates are host specific and safe to crops and native plants in Saskatchewan.

Six of the host-specific isolates were studied for infection under variable leaf-wetness (dew) duration at 20°C. Most of the isolates appeared to need at least 12 h of dew to cause visible disease. There were some slight differences among the isolates but the maximal infection generally occurred when 24 h of dew was provided. When compared to the similar pathogen from Europe, the indigenous strain 00-003B1 and 00-109A2 exhibited almost the same characteristics including the dew requirement for infection and the level of weed control.

Control of scentless chamomile was dramatically improved with a combined use of post-emergent herbicides and a strain of the host-specific fungus. Pretreatment with Basagran or Sencor 24 h before the inoculation with the fungus 99-21A_1 enhanced the level of weed control substantially compared to either the fungus or herbicide treatment alone. By itself, the fungus was only moderately effective, and so were the herbicides. The combination of Sencor and fungus reduced the plant fresh weight by approximately 98%, as opposed to 73% by the herbicide alone. This synergy resulted in 100% mortality of the weed one week after the treatment.

Often high spray volumes are required to achieve acceptable weed control by biocontrol organisms, making the strategy less practical for field applications. Studies were conducted to evaluate potential reduction of the carrier volume and improvement of biocontrol through adjustment of application parameters and the spore concentration. It was found that finer spray droplets improved the distribution or retention efficiency on scentless chamomile, potentially maximizing the coverage of targets. Opportunities exist in formulation for further improvements over distribution and retention

of microorganisms during applications. A high carrier volume may not be necessary if high spore concentrations can be used to deliver the same amount of pathogen inoculum to scentless chamomile. The isolate 00-003B1 applied in a higher spore concentration at 200 L/ha (90L/ac) achieved the same level of weed control as did runoff spraying (equivalent to over 2000 L/ha).

This project gathered useful background information about pathogen spectrum occurring on scentless chamomile in Saskatchewan and explored the potential of these pathogens as biocontrol candidates. By conducting systematic field surveys and evaluations, virulent and host-specific pathogens have been identified that cause damage to scentless chamomile but exhibit no negative effects to crop species. It is important to note that these indigenous isolates demonstrated a similar level of weed control as did their foreign counterparts from the area of weed origin in Europe. Further studies of these pathogens should be pursed to capture the value of this project and to develop the most promising candidates into potential biocontrol products. Formulation technologies will be the essential element for these pathogens to overcome moisture constraints under field conditions. The observed synergy between the biocontrol fungus and herbicides points towards a potential formulation and application strategy for enhanced weed control. In conclusion, we believe biocontrol can be an additional tool to improve the control of scentless chamomile in Saskatchewan.

Technical Report

Background and Objectives

Scentless chamomile, *Matricaria perforata* Mérat, is a noxious weed in Saskatchewan, Alberta and Manitoba, and a Class-3 secondary noxious weed under the Canada Seeds Act (Woo et al. 1991). The native distribution of *M. perforata* ranges from northern Spain, northern Italy and the central Balkan peninsula northwards to most of Scandinavia, eastwards into European Russia and the Caucasus, and parts of western Siberia and central Asia (Kay 1994). Scentless chamomile was introduced to the Canadian prairies from Europe at the beginning of the 20th century (Woo et al. 1991), and has been a very adaptable species which can grow either as a winter or summer annual, a biennial or a short-lived perennial (Hegi 1987, Woo et al. 1991). In Canada, it occurs in all provinces, but is most abundant in the Prairie Provinces and the Atlantic region. These two centers of abundance correspond to the distribution of two different cytotypes, diploid and tetraploid. The diploid type occurs primarily in the Atlantic region and the tetraploid on the prairie (Woo et al. 1991). The tetraploid type has developed into a serious weed problem on disturbed land on the prairies.

The weed has spread rapidly in recent years, especially in the Parkland region in the provinces of Saskatchewan and Alberta (Cole 1994, Bowes et al. 1994, Thomas et al. 1996). A single plant of scentless chamomile can produce up to a million seeds. A survey conducted by the coordinator of provincial Noxious Weed Management Program indicated that 66% of 156 RMs responded reported infestation by the weed (Figure 1). The heaviest infestations have been found around St. Walburg, Hafford, Shellbrook, Nipawin, Wynyard, Balgonie, and Esterhazy. On agricultural land it is particularly common around slough margins, in field depressions and along transition areas such as fence lines and right-of-ways (Bowes et al. 1994). In infested fields, scentless chamomile often becomes the worst weed problem due to the difficulty of control. This weed can cause serious yield losses in major prairie crops including spring wheat and forages (Douglas and Thomas 1991, Hoag et. al. 1980, Cole 1994). Losses can be more substantial in less competitive crops such as lentils, peas, and flax. In addition, this weed is a serious problem to rural municipalities, wildlife conservation areas, railroads and highways due to the cost and difficulty in managing it.

Scentless chamomile is a difficult weed to control in crop fields using conventional strategies. Most post-emergent herbicides are not sufficiently effective. A number of herbicides are registered for cereals, but the option is lacking on many other crops including pulses and forages (Saskatchewan Agriculture and Food 2001). In Alberta, of the nine herbicides registered in wheat, five are bromoxynil products (Ali 1995) that are effective only on seedlings of scentless chamomile, and plants that passed the 4-leaf stage generally can not be controlled effectively at crop-tolerant rates (Clark Brenzil, Saskatchewan Agriculture and Food, personal communication). The remaining herbicides are either marginally effective, restrictive on subsequent crops, or too expensive. In general, winter annuals of scentless chamomile are more competitive than summer annuals, and crop yield losses are greater in moist years due to excessive growth of the weed.

Tillage is only partially effective, especially under moist soil conditions or in heavy-soil fields, because the fibrous roots of scentless chamomile often adhere to large soil clumps and tillage often merely transplants the weed (Peschken et al. 1990). Mowing can reduce seed set but effective timing can be a challenge. Hand pulling is obviously impractical for large infested areas. Biological control using insects has been explored in Alberta and Saskatchewan (Bacher 1994, Hinz and McClay 2000, Peschken and Sawchyn 1993). A seed-feeding insect (*Apion hookeri*) and a stem miner (*Ceutorhynchus edentulus*) have been released to suppress the weed population in infested areas.

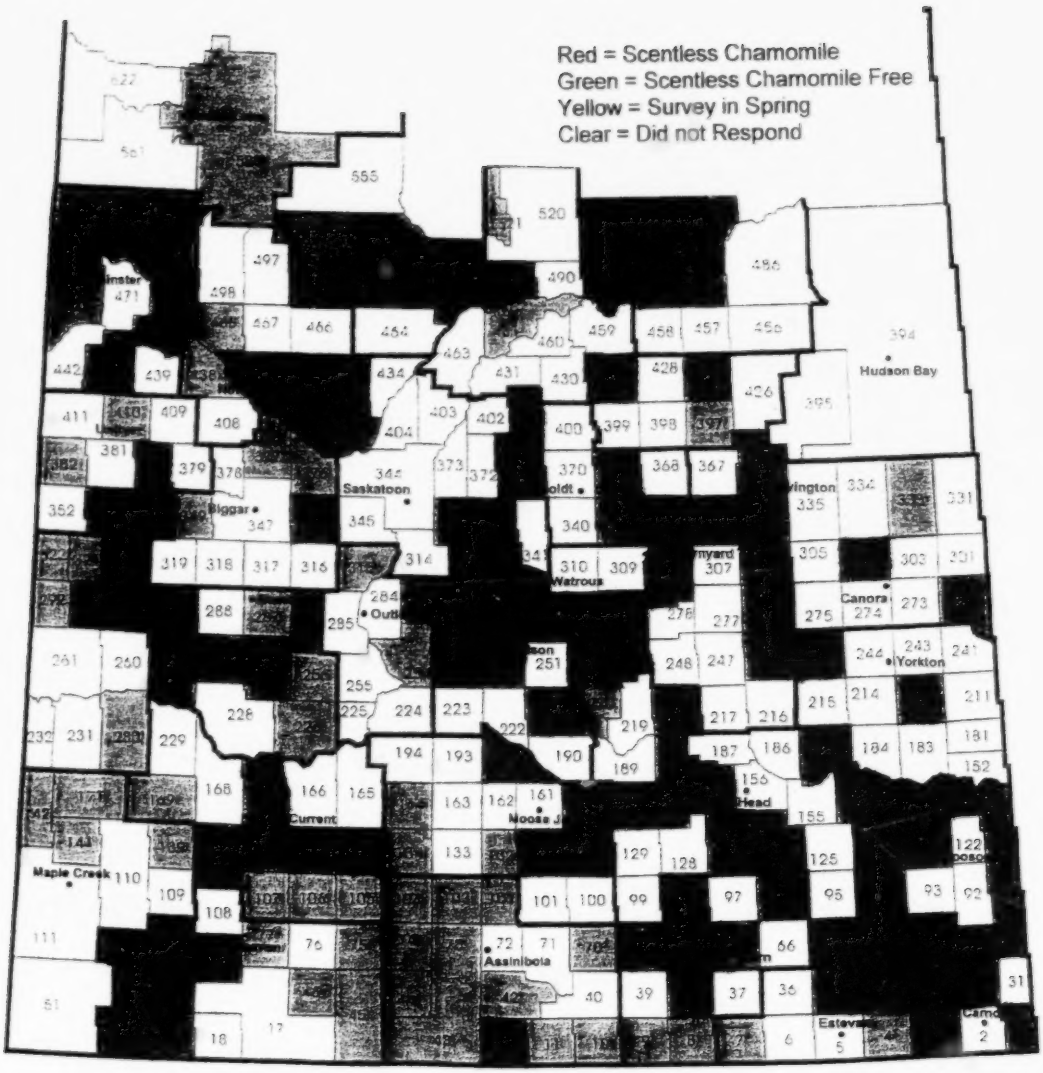


Figure 1. Infestation of scentless chamomile in Saskatchewan. The information is gathered by Dr. Gary Bowes (Coordinator of provincial noxious weed control program, Saskatchewan Agriculture and Food) according to a mail survey of weed inspectors with rural municipalities between 1999 and 2000.

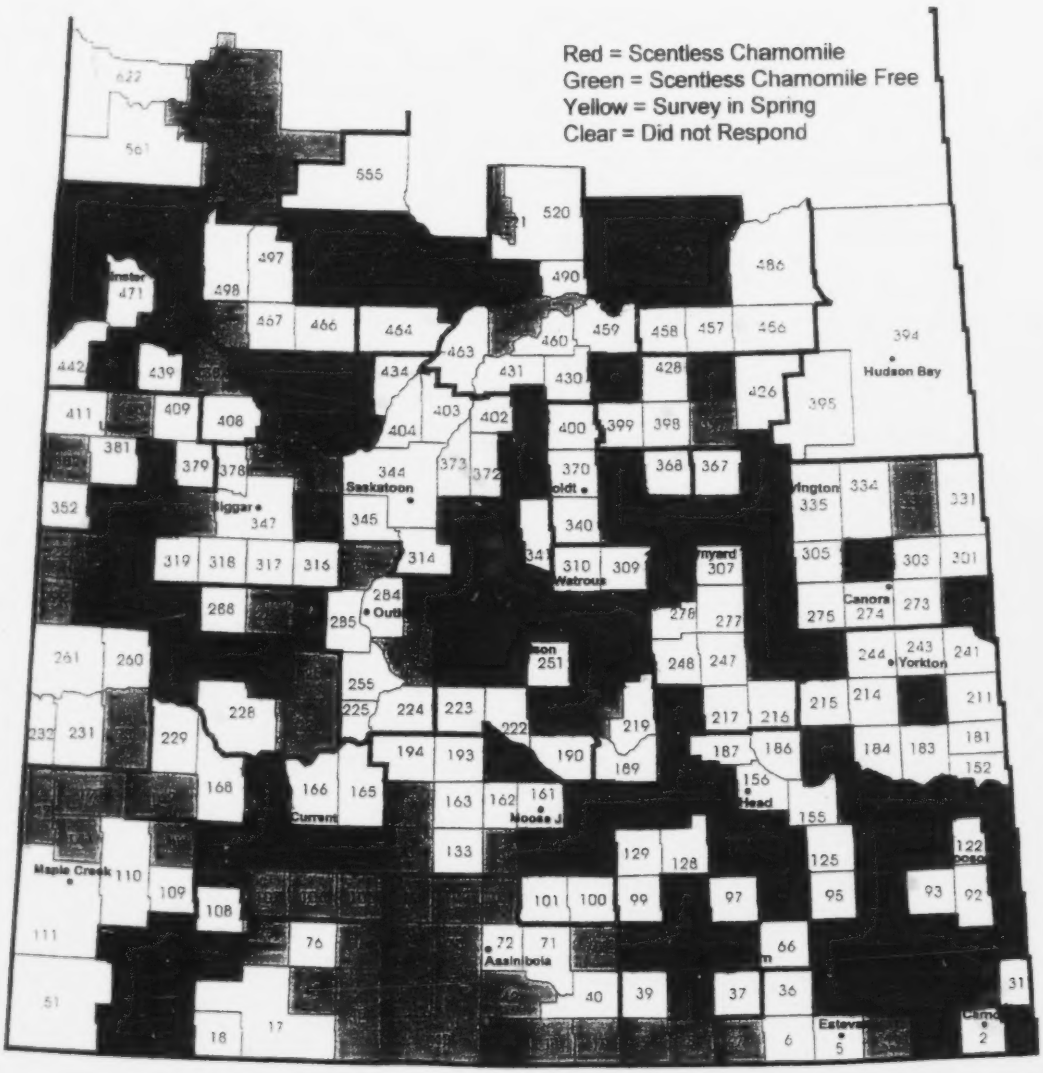


Figure 1. Infestation of scentless chamomile in Saskatchewan. The information is gathered by Dr. Gary Bowes (Coordinator of provincial noxious weed control program, Saskatchewan Agriculture and Food) according to a mail survey of weed inspectors with rural municipalities between 1999 and 2000.

These introduced insects may reduce the vigor or seed production to a degree but the short-term effect has been insignificant due to slow spread of these insects and only partial damage to the weed. Additional strategies are needed for more effective control of scentless chamomile.

Biological control using plant pathogens represents a potential new strategy. However, research information is generally lacking on microbial biocontrol, especially with inundative application of plant pathogens. A 'yellows-type' disease caused by a phytoplasma was found in some fields in Alberta (Khadhair et. al 1999), but the agent is not a suitable candidate for commercial development and its effectivity is rather limited since migrating insect vectors are required for spread of the disease. Preliminary field surveys in Saskatchewan have revealed several fungal pathogens causing disease on seedlings, but damage to older plants (over 7 leaves) was only moderate.

In the literature, a range of pathogens has been reported on scentless chamomile. In addition, scientists at CABI Bioscience Switzerland Centre have observed more diseases since 1994. Based on published information plus fungal collections held at CABI Bioscience [formerly known as the International Institute of Mycology (IMI)], a total of 31 fungal species have been associated with *M. perforata* and closely related species including *M. maritima, M. recutita* and *M. matricarioides* (Appendix 1). Many of them are plurivorous pathogens, attacking a group of plant species. Some are obligate parasites such as rusts and powdery mildews that depend on living tissues of plants. Several pathogens including *Ascochyta matricariae, Colletotrichum gloeosporioides, Fusarium oxysporum, Ramularia matricariae*, and *Septoria matricariae*, however, can be fairly host specific, infecting a relatively narrow range of plant species. Some host-specific fungal species of *Colletotrichum* and *Ascochyta* have been studied or developed as microbial herbicides (TeBeest and Templeton 1985, Mortentsen 1988, Vurro et al. 2001).

The above information indicates a possibility of finding a diverse group of pathogens on scentless chamomile. The objectives of this project were to conduct extensive field surveys in Saskatchewan to discover efficacious pathogens that cause infection and diseases on scentless chamomile and to evaluate the potential of these pathogenic organisms for biological control of the weed.

Materials and Methods

The project started off with extensive field surveys in the province to discover diseases on over-wintered and spring-emerged scentless chamomile and to isolate a range of potential pathogens. A bioassay using detached leaves of scentless chamomile was developed to verify the pathogenicity of the isolates. Pathogenic organisms were then applied as inundative foliar sprays to whole plants of scentless chamomile to evaluate their efficacy of weed control. Efficacious pathogens or isolates were subjected to studies of host specificity to ensure crop safety for field applications in western Canada. Host specific organisms were further studied for the dew requirement during infection and for practical applications using common spray equipment. Leading candidates were compared with ected exotic pathogens obtained through a parallel foreign exploration project.

Field surveys.

Surveys were conducted in the infested areas across the province between May and September for diseases of scentless chamomile at different growth stages or under variable weather conditions. In 2000, surveys were carried out regularly during the entire growing season, covering 39 RMs scattered around in brown to black soil zones. In 2001, field collection was focused on early season diseases from over-wintered weeds. The southeastern corner of the province was extensively surveyed because rainfall was frequent during spring. The collection sites represented diverse landscapes ranging from roadsides, ditches, pastures, to various crop fields. Individual plants of scentless chamomile were examined for disease symptoms. Diseased plants or tissues were placed in a cooler for later inspection in the lab. Pathogens were isolated from suspected disease tissues using standard protocols of plant pathology.

Pathogenicity bioassay

A bioassay was developed for rapid verification of pathogenicity. Detached leaves of scentless chamomile were surface sterilized using 10% Javex, then placed on moist filter papers in petri dishes. Mycelium plugs (about 5x5 mm) of purified fungal cultures were placed on the detached tissues, and the petri dishes were kept at the room temperature for infections from the plug inoculation. Four to six mycelium plugs were placed on 2 - 3 separate pieces of chamomile leaf tissues. The inoculated leaves were checked every 2-3 days for up to 14 days. Organisms that caused distinctive disease symptoms were considered potential pathogens and retained for further evaluation.

Screening for biocontrol efficacy

This process of screening is aimed at identification of effective organisms for control of established scentless chamomile.

Plants: seedlings were produced by planting seeds in Redi earth in plastic seeding trays. Individual seedlings were transplanted at 1 to 3-leaf stages into 3-inch pots containing a peat-based soilless mix amended with 3,500 g of 16-8-12 of N-P-K plus additional 700-g of 0-20-0 fertilizer in 3.8 ft^3 growth medium. Transplants were kept in the greenhouse for about 3 weeks until the 5 to 7-leaf stage.

Inoculum of fungal pathogens: All fungal pathogens were cultured on oatmeal or V8-juice agars at 25/20° C day/night temperature with 14 h of near-UV lighting for about 2 - 3 weeks or until a sufficient amount of spores was produced. To prepare the inoculum, fungal cultures were flooded with water containing 0.1% Tween 80 as surfactant, and rubbed with a bent glass rod to scrape off the spores. The concentration of the spore suspension was estimated with aid of a hamecytometer and adjusted to approximately 10^5 - 10^6 spores/ml.

Plant inoculation: Spore suspensions of various fungal pathogens were applied to the foliage of scentless chamomile (5-7 leaves) until runoff using an air-brush sprayer unless stated otherwise. Several plants were inoculated with each fungal isolate as replications, and water-treated plants were always included as controls various trials. Treated plants were placed immediately in a dew chamber for 24 hours to allow the spores to germinate and infect the plants, then kept in the greenhouse for two weeks to allow the development of disease. In one study in which spray retention was compared between different application methods, a cabinet sprayer was used with flat-fan Tee Jet nozzles to product practical droplet spectrum.

Efficacy assessment: Treatment effects were assessed according to disease severity at one and two weeks, and plant fresh weight at two weeks after inoculation. The disease severity was measured using a 0 to 5 scale in 2000, which represented a damage level from no disease to plant mortality. To facilitate the grading process and statistical analysis, a 0 to 6 pre-transformed rating scale (Little and Hill 1978), which corresponded to disease levels of 0, 0-7%, 7%-25%, 25%-50%, 50%-75%, 75%-93%, and 93-100% on scentless chamomile, was used in 2001. For fresh weight, plants were cut at the soil line and weighed individually for each replicate. Organisms causing significant disease symptoms or dramatic reduction of plant fresh weight were preserved in – 80°C ultra-low freezer for further evaluations.

Host-range studies

All pathogenic isolates that caused dramatic damage on scentless chamomile were tested for host-range to determine the crop safety. The pathogens were evaluated initially on 5 common crop species [wheat, canola (*Brassica rapa*), lentil, chickpea and flax]. In initial host-range testing, lentil and flax appeared to be very sensitive to several pathogens obtained from scentless chamomile. Isolates exhibiting no negative effect on these crops were subjected to expanded host-range test including 14 plant species belonging to the families *Asteraceae* (3), *Brassicaceae* (3), *Fabaceae* (4), *Poaceae* (3), and *Linaceae* (1) following the principle of centrifugal-phylogenetic procedure scheme by Wapshere (1974).

Transplants were used for scentless chamomile, ox-eye daisy and pineapple weed following the production procedure described above. Other host-range plants were seeded directly in Pro-Mix in 4 or 5" pots containing 5 to 10 plants/pot. The plants were kept in the greenhouse prior to inoculation. Spore suspensions of the fungal pathogens were applied using an airbrush sprayer to the foliage of all test plants till runoff. Conducive conditions, including high inoculum doses (5×10^5 to 1×10^6 spores/ml) and 48-h leaf wetness, were provided after inoculation to facilitate potential infection. Treated plants were kept in the greenhouse for at least 2 wk to allow for expression of disease symptoms. In addition, plant fresh weight was measured in comparison to those of water controls to ensure no negative effect on plant growth if latent infection occurred. Depending on species, plants were inoculated generally at 4 to 6-leaf stages. Three replicated pots were used for each plant species in a trial.

Minimal dew requirement for infection

Six fungal isolates that exhibited a high level of weed-control and host specificity were tested for minimal dew requirement at a favorable ambient temperature. The objective of this study was to use an additional selection criterion to further differentiate among the selected isolates for top biocontrol candidates. A reduced requirement for leaf wetness is a desirable trait under the relatively dry prairie climate. Plants of scentless chamomile were inoculated with spore suspensions at 1×10^6 spores/ml. Immediately after inoculation, plants were placed in a dew chamber set at 20° C for 0, 12, 16, 20 or 24 h to provide different leaf wetness duration (dew periods). Plants taken out of the dew chamber were placed in the greenhouse and assessed for disease damage a week after inoculation. For each isolate, three to four replicated pots were used at each dew period.

Comparison of leading agents with similar organisms from Europe

Assessment of a range of biological traits of selected candidates pointed to a group of fungal agents with potentially the same taxonomic designation. Similar organisms were also obtained from Europe, exhibiting comparable characteristics when evaluated in separate trials. This study was to compare two leading indigenous agents with 3 similar top-performing foreign pathogens on the minimum dew requirement and weed-control efficacy to determine candidates for potential development. For the comparison of dew requirements, many trials were conducted over a period of 6 months using study procedures similar to those described above. Dew temperature of 20° C was used for all trials with wetness duration ranging from 0 to 24 h. Statistical analyses were performed on each individual isolate with a focus on the initiation of disease symptoms and the peak of damage in relation to the wetness duration provided after the inoculation. The weed-control efficacy, on the other hand, was compared side by side in two repeated trials in the containment facility. Aqueous spore suspensions amended with 0.1% surfactant and adjusted to approximately 1 x 10^6 spores/ml were applied to potted plants of scentless chamomile at 2 growth stages (6 and 13-leaf), using the inoculation method described above. Treated plants were placed in a dew chamber at 20°C for 24 h, then kept in a growth cabinet for disease development. Disease severity was examined one and two weeks after inoculation, and the plant fresh weight was taken at the two-week rating. The experiment was a 2 x 5 factorial design with 4 replicated plants for each treatment.

Delivery of biocontrol agents

During initial assessment, spore suspensions of biocontrol agents were applied till runoff using an airbrush sprayer. This method of application, however, tends to spray excessive volumes (up to 3000L /ha), potentially exaggerating the capability of biocontrol candidates (Greaves et al. 2000). The run-off spraying covers shortcomings in retention and distribution associated with practical application volumes, and the coverage can be assured and retention generally maximized. Variable efficacy has been observed with biopesticide agents at reduced application volumes (Jones 1994, Smith and Bouse 1981). This study was to determine a required broadcast volume for achieving similar spray retention using common spraying equipment and parameters, and to ensure biocontrol effectivity with the broadcast spray under variable application volumes.

There were three parts in this study. First, the retention was measured for airbrush-runoff spray and broadcast sprays at variable volumes to estimate potential required broadcast volumes that render

equivalent retention as the airbrush spray. Six replicated plants of scentless chamomile at about 8-leaf stage were sprayed with a Rhodamine WT dye solution containing 0.1% surfactant (v/v) Tween 80, and the relative retention was estimated by washing plant tissues in ethanol and measuring the dye amount on a spectrophotometer (Wolf et al. 2000). Measurements were taken for the whole plant and retention amounts were calculated in µl dye/mg dry matter. Plastic petri dishes were placed beside the plants in the spray chamber to collect and determine actual broadcast spraying volumes (Wolf et al. 1997). In addition, several commercial nozzles were used to evaluate the impact of spray droplet spectrum on the retention efficiency that measures the amount of spray retained against the amount applied. Effect of travel speed was also studied using a cabinet sprayer set at 0.54 Km, 1.08 Km, and 2.16 Km per hr. Due to the facility limitation, higher speeds were not tested.

The second experiment was designed to compare weed-control efficacy of bioagents applied with both airbrush and broadcast sprays. The broadcast volume was calibrated to achieve similar retention as that produced with runoff-airbrush spray. The objective was to determine, at equivalent retention volume, whether the two application methods would result in similar weed control. In broadcasting spray, a single Tee Jet XR 8004 nozzle was used to ensure at least 2,000 L/ha application volumes. Two leading agents, 00-003B1 and 00-190A2, were used as a model system in this experiment. Spore suspensions at approximately 10^6 spores/ml were applied and each treatment consisted of 4 replicated plants. The level of weed control was compared with airbrush inoculation and non-treated controls.

The final part of this study was to evaluate weed-control efficacy using variable rates of a bioagent applied using a broadcast sprayer with two different nozzles and at one common field application volume (200 L/ha or 90 L/ac). Sprays were carried out using a cabinet sprayer fitted with Tee Jet XR8001 or XR8002 nozzle. The fungal agent 00-003B1 was used with final spore concentrations at approximately 10^7/ml (high), 5 x 10^6 (medium), and 10^6/ml (low), respectively. Treated plants were given 24-h dew at 20° C, then placed in the greenhouse. The efficacy was assessed 2 weeks after inoculation based on the disease severity and the plant fresh weight. Various pathogen treatments will be compared against non-treated controls.

Preliminary investigation of synergy between selected herbicides and biocontrol agents

Although the identified fungal pathogens caused significant suppression of scentless chamomile, weed mortality did not happen frequently with biocontrol treatment alone, especially on more mature plants. To enhance the efficacy, synergy was explored between bioagents and registered herbicides. A moderately effective bioagent, 99-21A_1, was used with two common broad-leaf herbicides, Basagran and Sencor. On plants over the 10-leaf stage, both bioagents and herbicides caused only moderate damage. To avoid potential compatibility issue, herbicides were applied at label rates 24 h prior to inoculation with the fungus. The efficacy of the combined use was compared with that of single treatment alone based on damage symptoms and fresh-weight reduction two weeks after inoculation.

Experimental design and data analysis

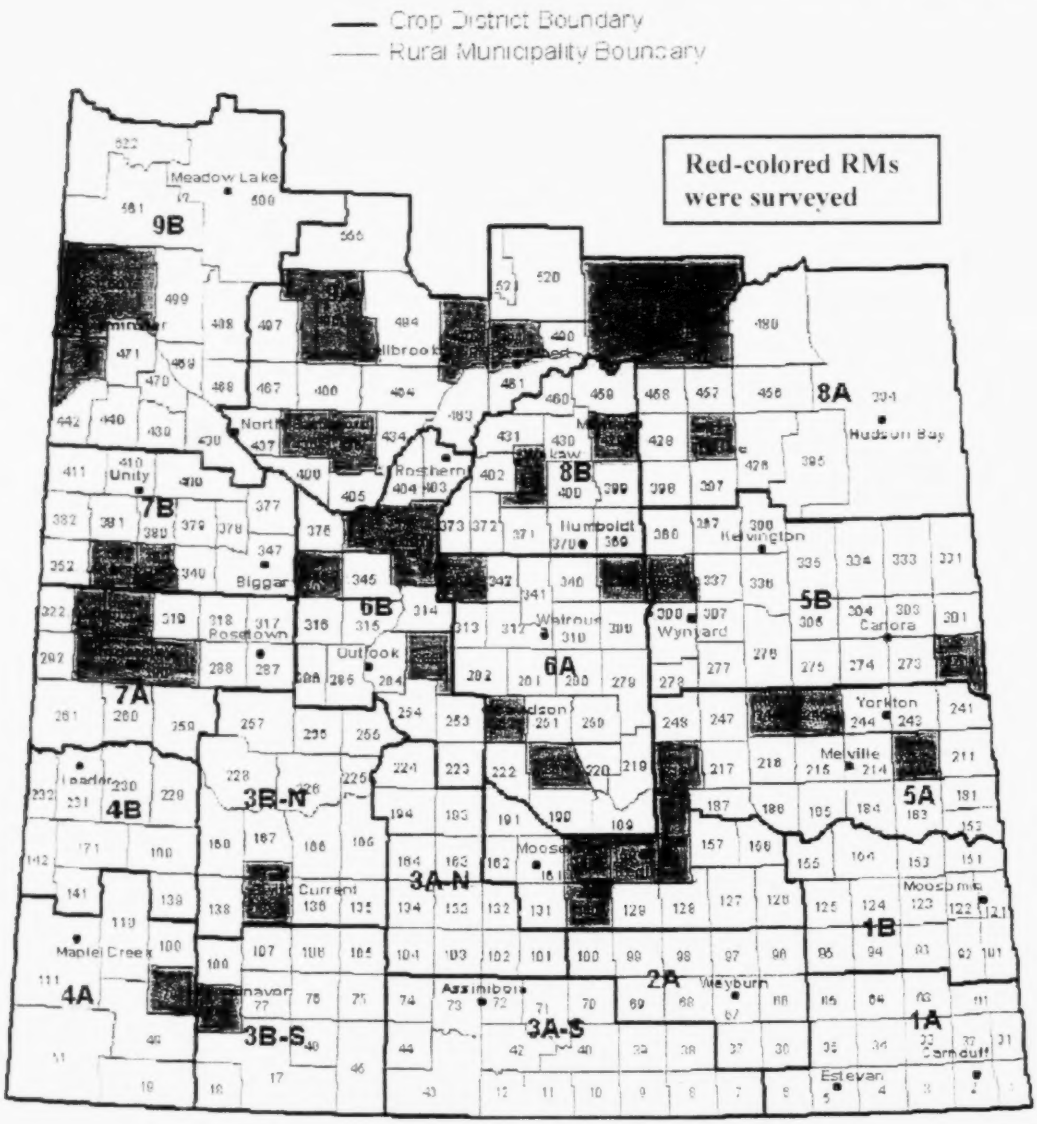
All experiments were repeated at least once after agents passed the efficacy screening stage, unless indicated otherwise. Completely randomized design was applied to all experiments. Results from repeated trials were pooled prior to analyses when their variances were homogenous, or otherwise analyzed separately. Data from most experiments were subjected to analysis of variance (ANOVA) and then multiple comparison of means. The $P < 0.05$ level of significance was used in all statistical procedures unless suggested differently.

Results and Observations

Field surveys

A part-time technician and a private contractor were hired to conduct the majority of survey activities during growing seasons of 2000 and 2001, including site visit, disease collection, and agent isolation. A significant amount of assistance was received from two AAFC technicians for field surveys and data analysis. In 2000, a total of 363 isolates were obtained from samples collected from 39 different RMs (Figure 2) where scentless chamomile had been previously reported. In 2001, diseases were seen less due to drought conditions. Field surveys focused more on 24 RMs around the southeastern corner where rainfalls were frequent during the growing season. Also, this area was not visited in 2000. A smaller number of disease samples were collected in 2001 with a total of 142 microbial isolates obtained. Over 95% of the microbial isolates obtained were fungi and only a few were bacteria. In two years, the survey covered the majority of sites where scentless chamomile had been reported, and a total of 505 microbial isolates were obtained. Description of field collection sites can

Figure 2. Rural municipalities surveyed in growing season of 2000 for disease-causal agents on scentless chamomile



Saskatchewan Agriculture and Food

0 50 100 150 Kilometers

N

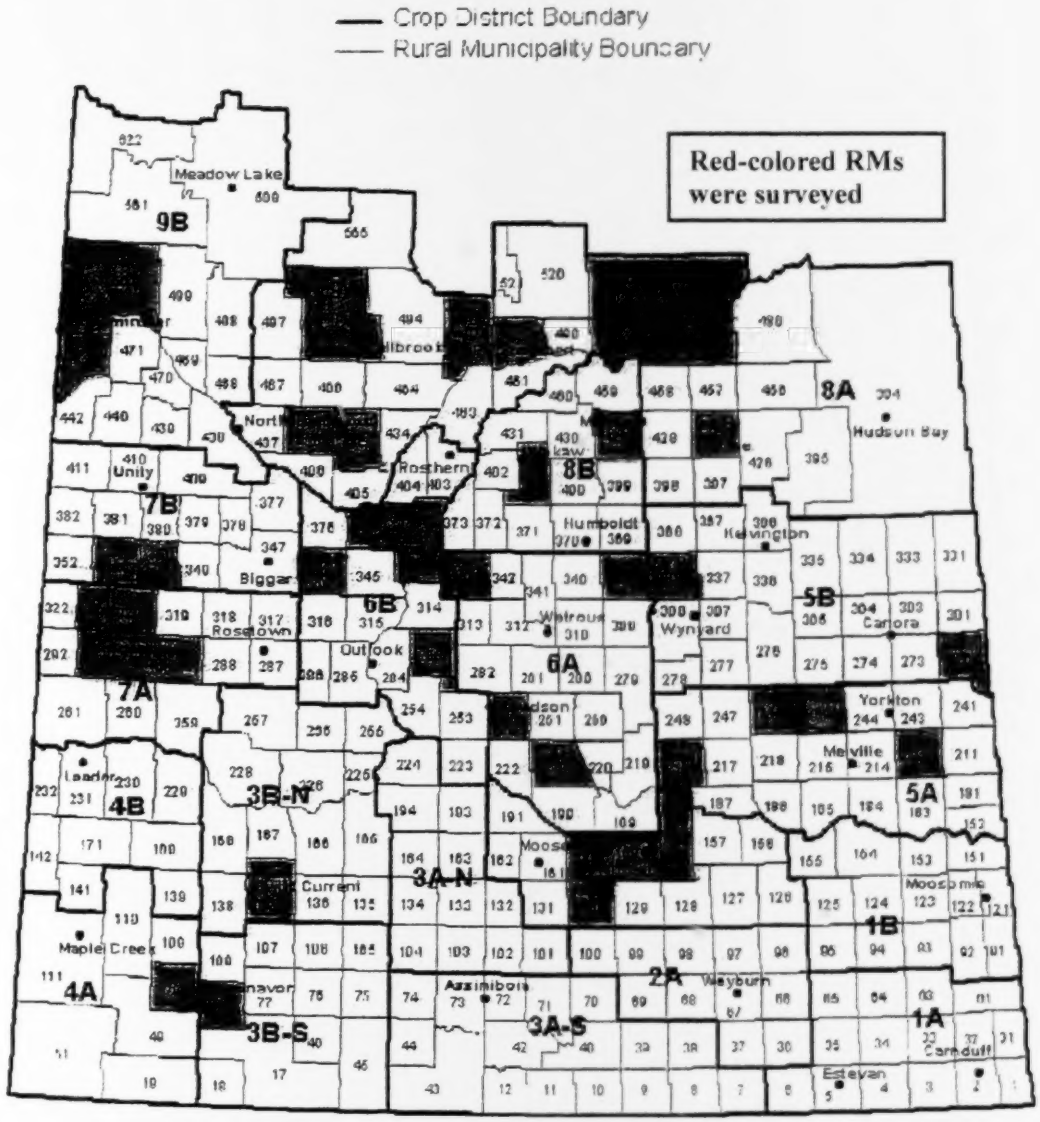
Prepared By: Geomatics Unit, Statistics Branch

Data source:

Date: June 7, 2000

©2000 Government of Saskatchewan

Figure 2. Rural municipalities surveyed in growing season of 2000 for disease-causal agents on scentless chamomile



©2000 Government of Saskatchewan

be found in Appendix 2 of this document. During field surveys, severe diseases were less frequently encountered on scentless chamomile. More often leaf spots were seen on young plants in early spring and late summer. It is speculated that the light disease is due to a relatively low level of natural pathogen inoculum.

Pathogenicity bioassay

Close to 500 purified microbial isolates obtained from diseased scentless chamomile were tested on detached chamomile leaves for pathogenicity. Among 363 fungal isolates obtained in 2000, 167 caused visible disease on detached tissues of scentless chamomile. As a result, they were retained for further testing. For the 142 isolates collected in 2001, 96 appeared to be pathogenic and were kept for biocontrol assessment. Based on bioassay results, approximately 50% of the isolates obtained were pathogenic. Disease symptoms on detached tissues were variable but necrosis appeared to be the most common type (Photo 1). In most cases, symptoms became visible within 2-wk incubation. Several organisms caused disease more rapidly, especially the ones isolated from over-wintered scentless chamomile early in the season. A total of 263 fungal isolates were identified as pathogenic and subsequently tested for weed-control efficacy. Isolates that caused no visible disease in the bioassay were autoclaved and discarded.

Screening for biocontrol efficacy

In two years, a total of 218 pathogenic isolates were tested for weed-control using foliar-inoculation method. These isolates, although exhibiting the pathogenicity on detached tissues, showed variable level of weed control on the whole plant of scentless chamomile ranging from highly effective to completely ineffective (Table 1). A significant number of isolates with mediocre disease symptoms showed slightly higher fresh-weight reduction possibly due to background variability of the plants. The weed control by individual candidate is detailed in a summary table in Appendix 3. Twenty-seven of the isolates that caused either severe disease or dramatic fresh weight reduction (close to 50%) on scentless chamomile were and kept for host range studies. Several isolates resulted in mortality of the weed at the 5 to 7-leaf stage under controlled conditions, reducing the plant fresh weight by up to 71% when compared to non-treated controls (Figure 3). This high level of weed control had not been seen with any chamomile pathogens collected previously. About 45 pathogenic isolates were not tested with the whole-plant system due to very little growth or sporulation on

different media tried. This poor growth or reproduction is considered to be disadvantageous for a biocontrol agent, therefore further evaluation of these organisms were not pursued.

Table 1. Control of scentless chamomile by pathogenic fungal isolates

Measurement (218 isolates tested)	**Distribution of candidate isolates based on the disease or fresh-weight reduction caused on whole plants**			
	0 – 25%	**25 – 50%**	**50 – 75%**	**> 76%**
Disease severity (%) *	204	7	4	3
Fresh-weight reduction (%) #	155	49	10	4

* The disease severity was measured using the 0 - 6 scale representing the diseased portion of a plant ranging from 0 to 100%.

\# The fresh weight of treated plants was compared against that of water controls.

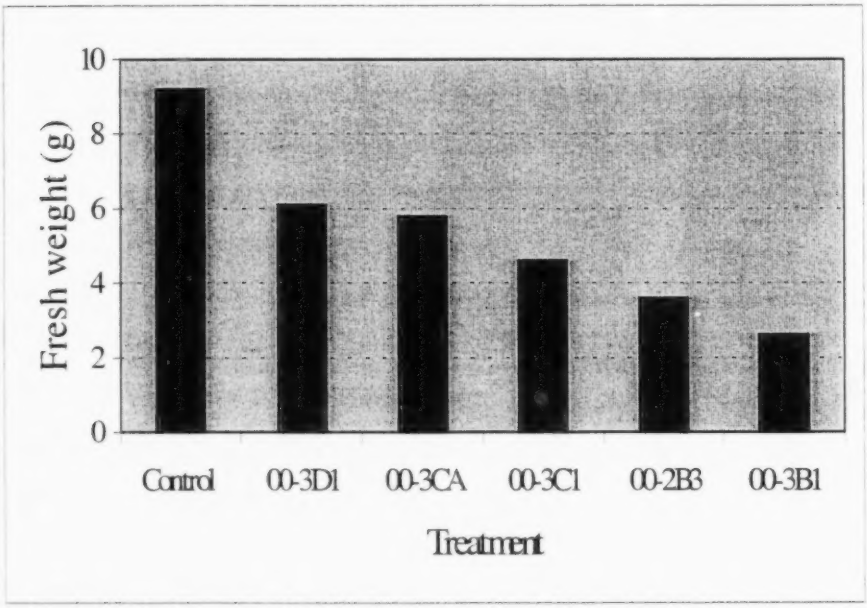


Figure 3. Reduction in fresh weights of scentless chamomile by selected fungal pathogens applied as an aqueous spore suspension to the weed at the 5 to 7-leaf stage.

Initial studies on crop safety

Initial host-range testing on 5 selected crop species revealed that 3 pathogenic isolates also caused significant disease on canola, pulse crops, and flax. These isolates were identified as *Fusarium sporotrichioides*, normally a weak pathogen on cereal crops. Members of this species are also notorious for mycotoxin production, although individuals may vary in their production ability. Serious T-2 and HT-2 toxins are most frequently associated with this species, limiting their potential to become a broadly applied biocontrol organism. Several other isolates, primarily Bipolaris spp. and Alternaria spp., caused noticeable disease on wheat, canola, lentil, or flax and were considered as potential crop pathogens as well. Comparatively flax and lentils appeared to be more sensitive than other crops to fungal infection (Table 2). However, thirteen isolates (species identify unknown) resulted in severe damage only to scentless chamomile. Host-range studies can be one of the most time-consuming procedures during evaluation of biocontrol agents due to potentially a large number of biocontrol candidates and testing plants involved. This initial test using representative crop species allowed eliminating detrimental crop pathogens quickly from the evaluation processes, enhancing the efficiency of the system.

Expanded host-range test

In this study, seven isolates that passed the initial testing were applied to a larger selection of plant species including common field crops and several closely related native plant species. Two isolates, 00-193C and 00-215B caused disease only on scentless chamomile (Table 3). Isolate 00-002B3 and 00-003B1 were also able to infect a closely related species, *Matricaria recutita* (German chamomile), although the disease severity appeared to be lower than that on scentless chamomile. In general, these isolates were considered to be very host specific. Isolate 00-248A1 and 00-190A2 resulted in disease symptoms on several crop species, but the damage was light in comparison to that caused by a real pathogen such as 00-079E that was damaging to almost every plant species tested. Considering the high dose of inoculum applied and extended dew period provided after inoculation, the testing conditions employed were likely extremely conducive for pathogen infection. Therefore the results can be strong indications that these biocontrol agents are safe to our crops and native plants. A couple of isolates caused disease on a closely related herb chamomile species (German chamomile), but they are not considered as a threat for field applications due to very limited acreage (less than 10 acres) and concentrated production (around Prince Albert) of German chamomile in the province.

Table 2. Pathogenicity of selected fungal isolates on common prairie crops (initial host-range test)

Species:	*Matricaria perforata*	*Cicer arentinium*	*Linum usitatissimum*	*Lens culinaris*	*Brassica rapa*	*Triticum aestivum*
Common Name:	Scentless chamomile	Chickpea	Flax	Lentil	Polish canola	Wheat
Cultivar:	N/A	Sanford	Vimy	Eston	AC Parkland	AC Crystal
00-002B3	+ *	–	+	–	–	–
00-003B1	+	–	–	–	–	–
00-003C1	+	–	–	–	–	–
00-003C4	–	–	+	–	–	–
00-063C2	+	+	+	+	+	–
00-79E	+	+	+	+	+	+
00-098B2	+	–	–	–	–	–
00-098C2	+	+	+	+	+	–
00-108C	+	–	+	–	–	–
00-111B	+	–	+	+	–	–
00-114E	+	–	+	–	–	–
00-190A2	+	–	+	–	–	–
00-193C	+	–	–	–	–	–
00-215A	–	–	–	+	–	–
00-215B	+	–	–	–	–	–
00-246A	–	–	–	–	–	–
00-248A1	+	–	–	–	–	–
00-262B	–	–	+	+	–	+
01-021A	–	NT #	NT	+	–	+
01-026B1	+	NT	NT	–	–	–
01-035B2	+	NT	NT	+	–	+
01-040	+	NT	NT	–	–	–
01-045A1	+	NT	NT	–	–	–
01-053A1	–	NT	NT	–	–	–
01-054A	–	NT	NT	–	–	–
01-060	+	NT	NT	–	–	–
01-080A1	+	NT	NT	+	+	+

* "+" and "–" represent disease symptoms present and absent, respectively.

\# NT = not tested

Table 3. Results of expanded host range testing

Species	Common Name	Cultivar	Isolate						
			00-079E	00-003B1	00-002B3	00-193C	00-215B	00-248A1	00-190A2
Matricaria perforata	Scentless chamomile	N/A	+*	+	+	+	+	+	+
Avena sativa	Oats	Calibre	+	–	–	–	–	–	–
Brassica juncea	Mustard	Cutlass	+	–	–	–	–	–	+
Brassica napus	Argentine canola	Westar	+	–	–#	–	–	–	–
Brassica rapa	Polish canola	AC Parkland	+	–	–	–	–	–	+
Chrysanthemum leucanthemum	Ox-eye daisy	N/A	+	–	–	–	–	–	–
Cicer arentinium	Chickpea	Sanford	+	–	–	–	–	–	–
Hordeum vulgare	Barley	Harrington	+	–	–	–	–	–	–
Lens culinaris	Lentil	Eston	+	–	–	–	–	+	+
Linum sitatissimum	Flax	Vimy	+	–	–	–	–	+	+
Matricaria recutita	German chamomile	N/A	NTζ	+	+	–	–	–	+
Matricaria matricariocoides	Pineapple-weed	N/A	+	–	–	–	–	–	–
Medicago sativa	Alfalfa	Beaver	+	–	–	–	–	–	–
Pisum sativum	Field pea	Alfetta	+	–	–	–	–	–	–
Triticum aestivum	Wheat	AC Crystal	+	–	–	–	–	–	–

* "+" and "–" represent disease symptoms present and absent, respectively.

\# One lesion on one plant only

ζ NT = not tested

Minimal requirement of leaf wetness for infection

Six isolates belonging to the same fungal genus were tested in this study. All isolates demonstrated high efficacy of weed control and general safety to our crops. At 20°C, these isolates behaved quite similarly in terms of the requirement for leaf wetness during the infection process. Based on the onset of disease symptoms, most of the isolates appeared to need at least 12 h of dew to cause visible disease (Figure 4), but the infection was sparse with little damage to plants. The infection advanced

with dew duration and the fastest increase appeared to occur between 20 and 24 h of dew, reflected by a dramatic rise in disease severity with several isolates. In comparison to the disease caused with shorter dew periods, disease severity was generally highest when 24 h of dew was provided. There were some slight differences among the isolates. Agents 00-193C and 00-215B were able to cause a low level of disease with 12 to 16 h of dew, while 00-002B3 and 00-248A1 needed at least 16 to 20 h to result in the same level of disease. With 24 h of dew, agents 00-002B3, 00-003B1, 00-193C, and 00-190A2 resulted in more effective and consistent weed control than did by others.

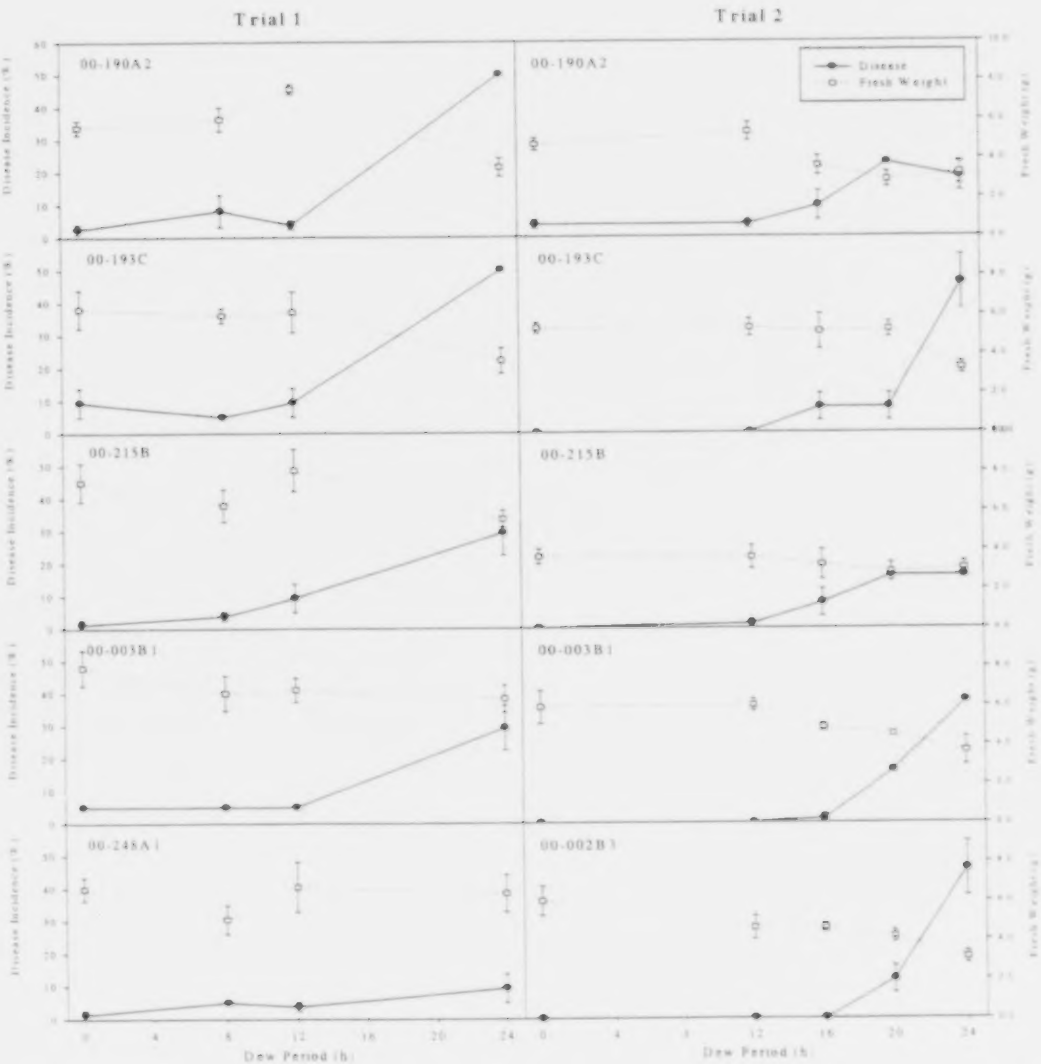


Figure 4. Effect of leaf-wetness duration on infection of scentless chamomile and weed-control efficacy by six indigenous fungal pathogens.

Comparison of leading agents with similar organisms from Europe

In comparison to several similar fungal isolates obtained from Europe, indigenous agents 00-003B1 and 00-109A2 exhibited almost the same biological characteristics including weed-control efficacy and dew requirement. In side by side trials, the indigenous candidates were equally effective as their foreign counterparts in suppression of scentless chamomile under controlled environment (Figure 5), reducing plant fresh weight by close to 70% in comparison to non-treated controls. The efficacy was similar on plants with either 6 or 13 leaves. Foreign isolate 01-21H-1 was noticeably less effective, especially on the older plants of scentless chamomile. Based on morphological and host-range characteristics, these indigenous and foreign isolates are considered belonging to the same fungal species. In general, all of them require 20 to 24-h dew to cause maximal damage to scentless chamomile.

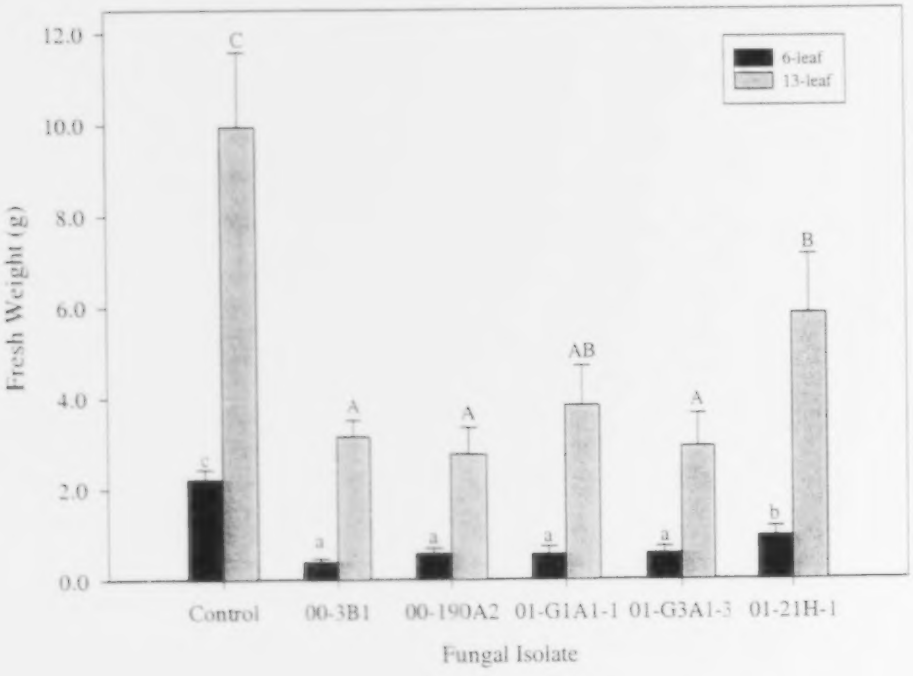


Figure 5. Weed control by two indigenous fungal pathogens (00-003B1 and 00-190A2) in comparison to three similar foreign pathogens on scentless chamomile of different growth stages.

Application methods for delivery of biocontrol agents

Extremely high application volumes are required to achieve an equivalent retention level produced by runoff airbrush spraying. Based on retention calculations, we would have to broadcast more than 2000 L per ha to retain a similar amount of spray on scentless chamomile as with the airbrush spray. Obviously, this is not practical. Several factors may affect the retention efficiency including spray parameters. In general, we found travel speed had little effect (Figure 6), although the faster speeds seemed to result in slightly lower retention. This observation is consistent with our previous report on green foxtail (Peng et al. 2001). At the same speed, finer droplets definitely enhanced the retention efficiency and the finest droplet spectrum produced with XR8001 nozzle generally gave the highest retention. Spray droplet sizes can be optimized to maximize coverage of targets (Jones and Berges 1998). However, for biocontrol agents there are limitations with extremely fine sprays due to the size of organism propagules and potential spray drift concerns (Jones 1998, Grover et al., 1997). To obtain further improvements, greater emphasis should be directed to formulations for better retention and distribution of microbial agents (Greaves et al. 2000).

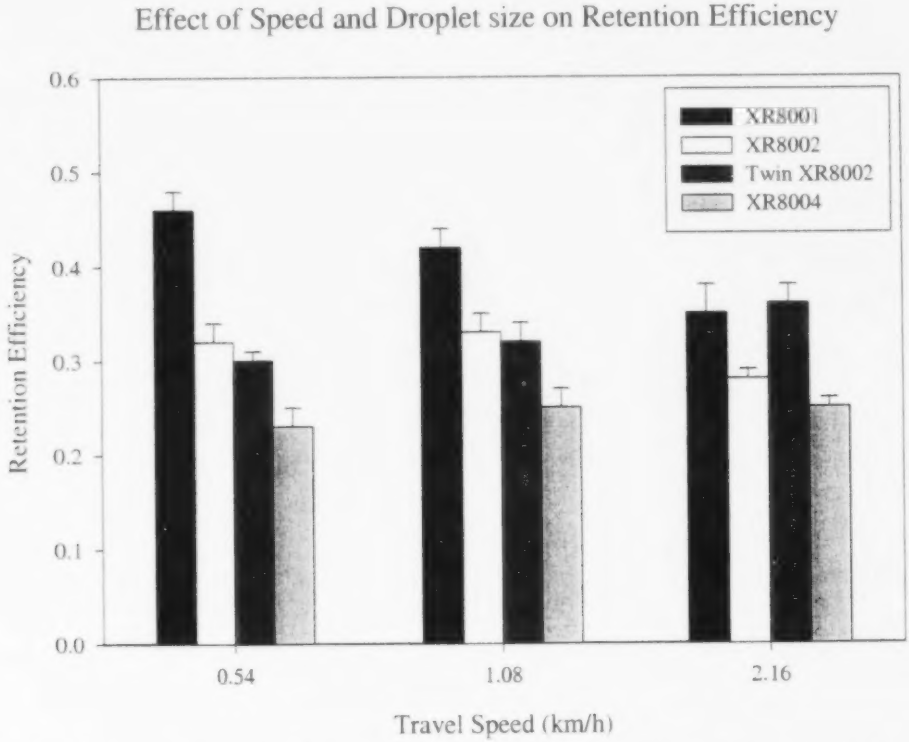


Figure 6. Spray retention efficiency on scentless chamomile applied using different nozzles and at variable travel speeds.

When high volumes were applied (over 2,000 L/ha) using a broadcast sprayer, similar weed-control efficacy was achieved as did the runoff-airbrush spraying (Figure 7). The broadcasting volume was calculated based on equivalent retention obtained with the airbrush spray. The plant fresh weight was reduced by approximately 50% by both application methods in comparison to the non-treated control. It appears that the retention volume is the key to biocontrol efficacy regardless off application methods used. Unsatisfying results associated with broadcast spraying may have more to do with poor retention resulting from low application volumes. In essence, it affects the actual rate of biocontrol agents applied. The next experiment looked at the possibility of using low application volumes through increasing the concentration of fungal inoculum.

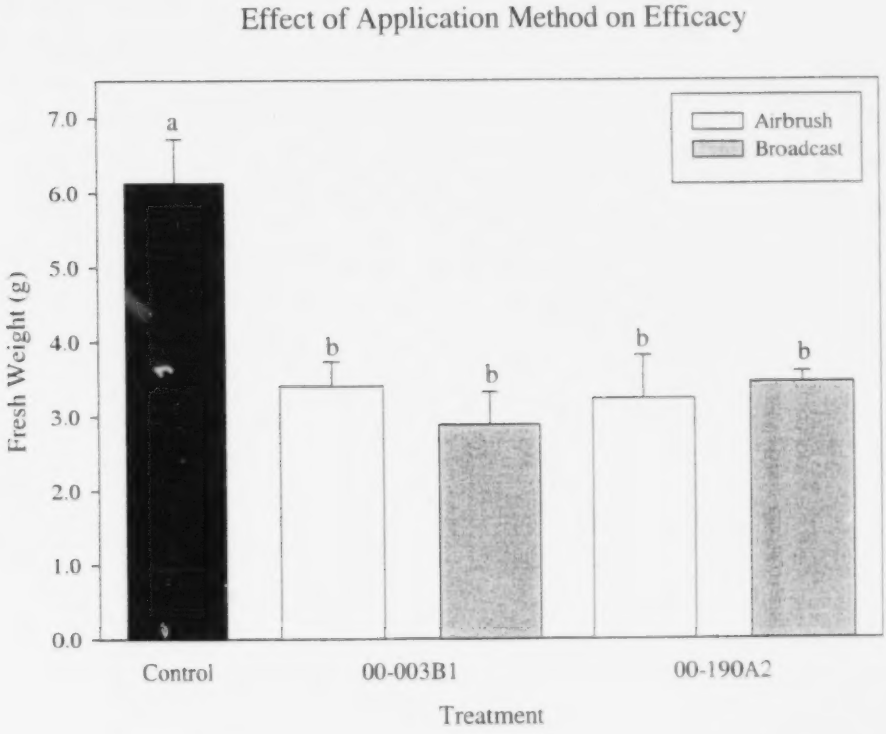


Figure 7. Control of scentless chamomile by two fungal isolates applied using a broadcast sprayer (at 2,000 L/ha) or airbrush sprayer (till runoff) that resulted in similar retention volumes on the plant foliage.

When applied at 200 L/ha (90L/ac) using a broadcast sprayer, the weed-control efficacy generally increased with the spore concentration used (Figure 8), a circumstance likely relating to the rate applied. In other words, it is the rate of fungal inoculum and not carrier volume that governs the efficacy. Similar effects were observed with the droplet spectrum. The XR8001 nozzle achieved slightly better weed control than did the courser nozzle XR8002. In general, there was little difference in the efficacy between broadcast and airbrush sprays, especially when finer-droplet nozzles were used. The use of high spore concentration can potentially reduce application volumes dramatically without compromising weed control. In addition, it increases the number of fungal propagules contained in the spray, reducing the number of 'empty' droplets (Jones 1998). Concentration, carrier volume, and droplet sizes need to be further evaluated with formulation to optimize the effectivity, practicality, as well as the cost for the use of biological control.

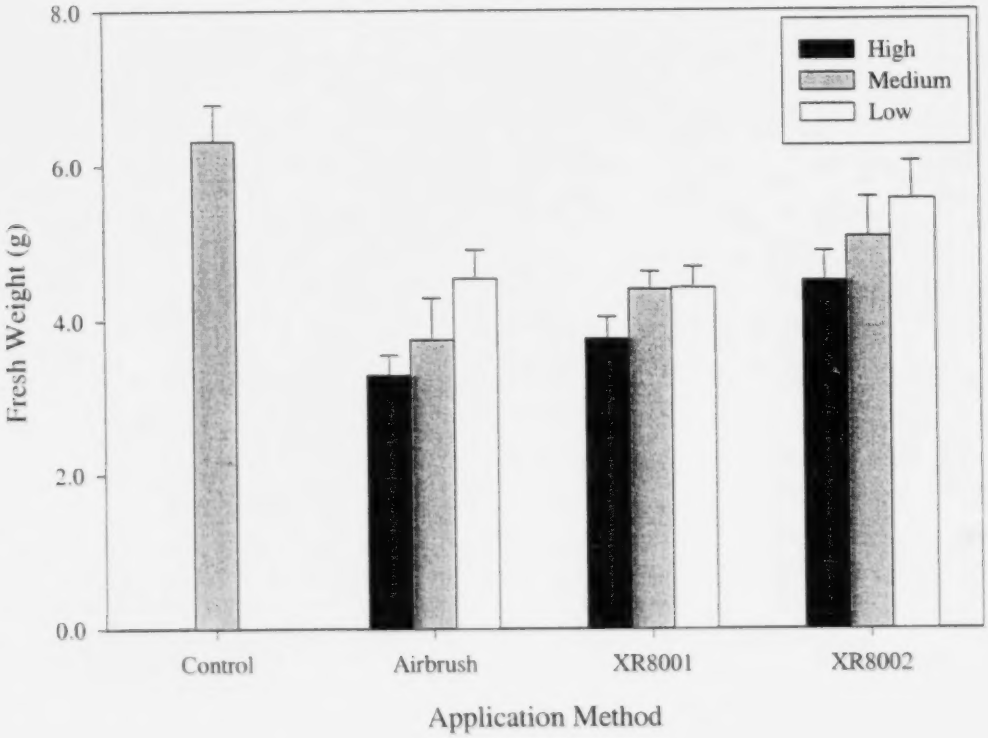


Figure 8. Control of scentless chamomile by 00-003B1 applied using a broadcast sprayer at a common field-application volume (200 L/ha) and 3 spore concentrations (High, Medium, and Low) in comparison to airbrush sprays until runoff.

Preliminary investigation of synergy between selected herbicides and biocontrol agents

Pretreatment with Basagran or Sencor 24 h before the inoculation with the biocontrol agent 99-21A$_1$ enhanced the level of weed control when compared to either the fungus or herbicide treatment alone (Figure 9). The fungus was only moderately effective by itself, as were the herbicides. It was apparent that plants of scentless chamomile at 10 leaves had already developed resistance to the herbicides. In comparison to non-treated controls, the combination of Basagran and fungus reduced the plant fresh weight by 62.9%, as opposed to only marginal damage caused by the herbicide alone. Sencor appeared to be more synergistic with the fungus, reducing the fresh weight by close to 98%, as opposed to 73% by the herbicide only. This synergy resulted in 100% mortality of the weed at about one week after the treatment (Photo 9).

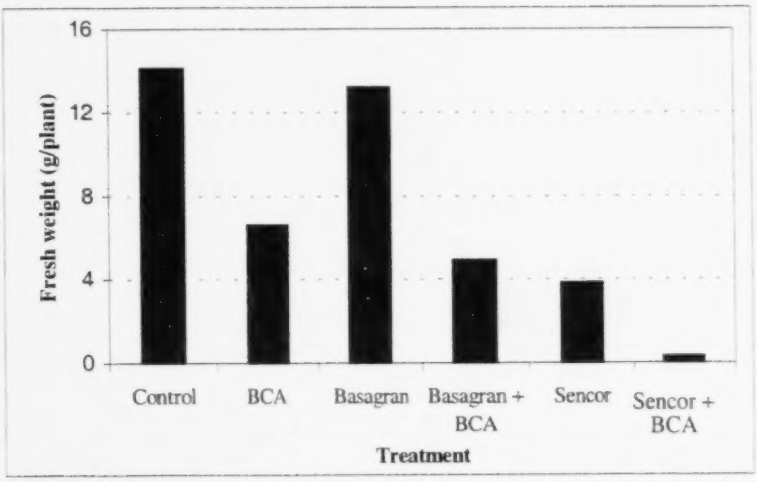


Figure 9. Efficacy of weed control by biocontrol agent (BCA= isolate 99-21A1), herbicides, and combination of herbicide-biocontrol agent.

Conclusions and Comments

Extensive field surveys in 2000 and 2001 yielded a large number of disease samples and over 500 microbial organisms were obtained for evaluation against scentless chamomile. In general, diseases were not common on the weed, especially on more mature plants. Sporadic leaf spots occurred more often on summer or over-wintered young plants. However, disease damage was generally low with natural infections, presumably due to less abundant pathogen inoculum and sub-optimal infection conditions. Collaboration with MRJ Biosurveys, a private business based in Balgonie, SK, enabled us to cover a large portion of the cropping areas in the province.

It is important to note that several host-specific fungal pathogens have been identified that cause significant damage to scentless chamomile in a controlled environment and have no negative impact on any of the field crops tested. These pathogens are considered promising due to several features they exhibited. They were effective against scentless chamomile up to 10-13 leaf stages, with the potential for a wide window of application. Some of them interacted with herbicides synergistically, resulting in enhanced control of scentless chamomile. In comparison to foreign isolates, they showed similar weed control and other biological traits. Using indigenous isolates for the development of microbial herbicides has fewer regulatory hurdles. We have also confirmed that these agents can be applied using common spray equipment with practical carrier volumes without compromising the weed control. Once validated in the field, a delivery system can be readily available for large-scale application.

Based on the results achieved in this project, we believe this research ought to be continued with more focus on the promising agents and critical technologies that assist the delivery and performance of these agents in specific crop systems, such as pulses, flax, and forage seeds. Combined, these crops exceed 2.5 M ha in Saskatchewan alone, representing significant economic value. In the foreseeable future, transgenic herbicide-resistant varieties will unlikely be available for these crops. Therefore, microbial agents can potentially be new tools in the integrated weed management system for these specialty crops, and also help organic producers, municipalities, and provincial highway departments in combating chamomile problems.

The synergy with herbicides can potentially increase the consistency of weed control by microbial agents while reducing the overall use of synthesized chemicals. Tank mixing of host-specific fungal pathogens with herbicides can be practical and efficient for synergistic applications. This research also contributes to the development of a new integrated management approach that may prove to be useful for addressing other challenging weed problems such as leafy spurge, kochia, wild buckwheat, and Russian thistle on the prairies. These weeds often are not adequately controlled with current strategies that depend heavily on chemical herbicides.

Biological control of scentless chamomile benefits rural communities at large. Under the Noxious Weed Act, rural municipalities are required to maintain the control of the weed along municipal

roads. Saskatchewan Highways and Transportation spends approximately a quarter of million dollars each year primarily on scentless chamomile along provincial highways. Additionally, the amount of chemicals used by CN and CP Railways on their rights-of-way and by contractors around waste land and gravel pits adds to the concern of the general public and rural residents about the pesticide load in the environment. An effective biocontrol component in these programs will not only increase the effectiveness of weed control but also help cut down pesticide usage.

Eventually, these biocontrol agents and associated technologies will have to be evaluated by private sector clients for the feasibility of commercial development. It is believed that small companies are better positioned to respond to advances in bioherbicide technologies. For this reason, microbial herbicides will likely be developed mostly for niche market opportunities in the near future. In Canada, several companies can potentially be commercial developers when this technology is ready for it. Philom Bios Inc. is a Saskatoon-based biological company specializing in microbial legume inoculant and phosphate-solublizing products on a range of crops in western Canada. MicroBio RhizoGen is also a producer/marketer of legume inoculant in Saskatoon that also has strong presence in western Canada. In addition, they are engaging in research and development of biofungicides to be used for the control of soil-borne diseases. Both companies have a history of collaboration with AAFC on several biocontrol projects, and possess expertise in commercial microbial fermentation and formulation. A new microbial herbicide for the control of scentless chamomile can be a valuable addition to their core business due to the synergy of targeting the same market sector.

To enhance the probability of private investment, some of the critical risk factors associated with the technology such as mass production, formulation, and delivery systems ought to be addressed. The progress in these aspects, combined with consistent weed control in field conditions, will help lower development risks, making the project more attractive for commercial venturing. At this point, continued support from ADF will be vital to development, validation, and demonstration of this technology.

Literature cited

1. Ali, S. 1995. Crop Protection with Chemicals. Agdex 606-1. Alberta Agriculture, Food and Rural Development, Edmonton, Alberta, Canada.
2. Bacher, S. 1994. Strategies for biological control of scentless chamomile in Canada. Zeitschrift*fuer Pflanzwnkrankheiten*-und-Pflanzwnschuts. Sonderheft. Spec. 14: 221-230.
3. Blumer, S. 1967. Echte Mehltaupilze (Erysiphaceae). Ein Bestimmungsbuch für die in Europa vorkommenden Arten. Fischer, Jena. 436 pp.
4. Bows, G.G. 1989. Scentless chamomile control program: Final report to ADF. 20 p.
5. Bowes, G.G., Spurr, D.T., Thomas, A.G., Peschken, D.P., & Douglas, D.W. 1994. Habitats occupied by scentless chamomile in Saskatchewan. Can. J. of Plant Sci. 74: 383-386.
6. Brandenburger W. 1985. Parasitische Pilze an Gefässpflanzen in Europa. Gustav Fischer Verlag Stuttgart & New York. 1248 pp
7. Buhr, H. 1964. Bestimmungstabellen der Gallen (Zoo- und Phytocecidien) an Pflanzen Mittel- und Nordeuropas. I. Fisher, Jena. 761 pp.
8. Butler Sir E. J. and Jones S. G. 1949. Plant Pathology. Macmillan & Co. Ltd, London. 206 pp.
9. Cole, D. 1994. Scentless Chamomile: Biology and Control. Alberta Agriculture, Food and Rural Development, Agdex 640-5.
10. Docters van Leeuwen, W.M. 1957. Gallenboek. Nederlandse door dieren en planten veroorzaakte Gallen. Thieme, Zutphen. 332 pp.
11. Douglas, D.W., Thomas, A.G. et al. 1991. Effect of summer and winter annual scentless chamomile interference on spring wheat yield. Can. J. Plant Sci. 71: 841-850.
12. Ellis M. B. and Ellis J. P. 1987. Microfungi on land plants. An Identification Handbook. Slough, England RP cop. 868 pp.
13. Farr D.F., Bills G.F., Chamuris G.P., Rossmann A.Y. 1989. Fungi on plants and plant products in the United States. St. Paul, Minnesota APS Press, 1252 pp.
14. Gäumann E. 1959. Die Rostpilze Mitteleuropas. Mit besonderer Berücksichtigung der Schweiz. Büchler & Co., Bern. 1407 pp.
15. Ginns J.H. 1986. Compendium of Plant Disease and decay of Fungi in Canada 1960 – 1980. Agric. Canada Publ. 1813. 132 pp.
16. Grove, W.B. 1935. British Stem- and Leaf-Fungi 1: 1-448. C.U.P.
17. Greaves, M.P., Duton, L. and Lawrie, J. 2000. Formulation of microbial herbicides. *Aspects of Applied Biology* 57 (Pesticide Application): 171-178.
18. Grover, R., Caldwell, B.C., Maybank, J, and Wolf, T.M. 1997. Airborne off-target losses and ground deposition characteristics from a Spra-Coupe using 'low drift" nozzle tips. *Can. J. Plant Sci.* 77:493-500.
19. Hegi, G. 1987. Illustrierte Flora von Mitteleuropa. Spermatophyta, Band VI, Angiospermae, Dicotyledones 4. Paul Parey, Berlin, Hamburg.
20. Hinz, H.L. and McClay, A. 2000. Ten years of scentless chamomile: prospects for the biological control of a weed of cultivated land. In: Neal R. Spencer [ed.], Proceedings of the X International Symposium on Biological Control of Weeds, 4-14 July 1999, Montana State University, Bozeman, Montana, USA, pp. 537-550.
21. Hoag, B.K., Lukach, J.R., Fisher, J.J. and Miller, S.D. 1980. False chamomile control in north central North Dakota. Proc. North Central Weed Control Conf. 35:86.
22. Horst R. K. 1979. Westcott's Plant Disease Handbook. Fourth Edition. Published by Van Nostrand Reinhold Company, New York. 803 pp.
23. Jones, K. A. 1994. Use of baculoviruses for cotton pest control. In: *Insect Pest of Cotton* (eds. G.A. Matthews and J. P. Tunstall), pp. 477-504. CAB International, Wallingford, UK.
24. Jones, K.A. 1998. Spray application criteria. In: *Formulation of Microbial Biopesticides* (ed. H.D. Burges), pp. 367-375. Academic Press, London.
25. Jones, K.A and Burges, H.D. 1998. Technology of formulation and application. In: *Formulation of Microbial Biopesticides*, pp. 7-30.
26. Kay Q. O. N. 1994. *Tripleurospermum inodorum* (L.) Schultz Bip. J. Ecol., 82: 681 – 697.
27. Kessler, D. 1989. Scentless Chamomile Series #3: Distribution. Canada/Saskatchewan Subsidiary Agreement on Agriculture Development, p 2.
28. Khadhair, A.H., McClay, A.,Hwang, S.F., and Shah, S. 1999. Aster yellows phytoplasma identified in scentless chamomile. Journal of Phytopathology. 1999. 147: 149-154.
29. Mortensen K. 1988. The potential of an endemic fungus, *Colletotrichum gloeosporioides* f. sp. *malvae*, for biological control of round-leaved mellow (*Malva pusilla*) and velvetleaf (*Abutilon theophrasti*). Weed Sci. 36:473-478.

30. Peng, G., T.M. Wolf, K.N. Byer, and B. Caldwell. 2001. Spray retention on green foxtail (*Setaria viridis*) using airbrush and broadcast sprayers and its impact on the efficacy of a mycoherbicide agent. Pp. 699-706 In: Proc. of 18th Asian-Pacific Weed Science Conference, by H.W. Ni and Z.G. You (eds.), June 2001. Beijing, China.
31. Peschken, D.P. & Sawchyn, K.C. 1993. Host specificity and suitability of *Apion hookeri*, a candidate for biocontrol of scentless chamomile. Can. Entomologist. 125: 619-628.
32. Peschken, D. P., A. G. Thomas, G. G. Bowes, and D. W. Douglas. 1990. Scentless chamomile (*Matricaria perforata*) - a new target weed for biological control. In: Proceedings of the VII International Symposium on Biological Control of Weeds, 6-11 March 1988, Ist. Sper. Patol. Veg. (MAF), Rome, Italy. pp. 411-416.
33. Saskatchewan Agriculture and Food. 2000. Guide to crop protection – weeds, plant diseases and insects. Saskatchewan and Manitoba Bi-Provincial Publication. 330 pp
34. Smith, D. B. and Bouse, L. F. 1981. Machinery and factors that affect the application of pathogens. In: *Microbial Control of Pest and Plant Diseases*, pp. 635-653.
35. TeBeest, D.O. and Templeton, G.E. 1985. Mycoherbicides: progress in the biological control of weeds. Plant Dis.69:6-10.
36. Thomas, A.G., Leeson, L.Y., Wise, R.F., and Juras L. 1996. Saskatchewan weeds survey: cereal, oilseed and pulse crops 1995. AAFC, Saskatoon Research Center. 419 p.
37. Vurro, M., Zonno, M. C., Evidente, A., Andolfi, A., and Montemurro, P. 2001. Enhancement of efficacy of *Ascochyta caulina* to control *Chenopodium album* by use of phytotoxins and reduced rates of herbicides. Bio.Control. 21:182-90
38. Wapshere, A.J. 1974. A strategy for evaluating the safety of organisms for biological weed control. *Ann. Appl. Biol.* 77:201-211.
39. Wolf, T.M., Liu, S.H., Caldwell, B.C., and Hsiao, A.I. 1997. Calibration of greenhouse spray chambers - the importance of dynamic nozzle patternation. *Weed Technol.* 11:428-435.
40. Wolf, T.M., Harrison, S.K, and Hall, F.R. 2000. Optimizing postemergence herbicide deposition and efficacy through application variables in no-till. *Weed Science* 48:761-768.
41. Woo, S. L., A. G. Thomas, D. P. Peschken, G. G. Bowes, D. W. Douglas, V. L. Harms, and A. S. McClay. 1991. The biology of Canadian weeds. 99. *Matricaria perforata* Mérat (Asteraceae). Can. J. Plant Sci., 71: 1101-1119.

Project-related Publications/presentations

Conference abstracts/posters

1. Peng G., K.L. Bailey, and K.N. Byer. 2000. Potential for control of *Matricaria perforata* with fungal pathogens plus registered herbicides. Proc. 5th International Bioherbicide Workshop (Abstr.) P12. Iguassu Falls, Brazil.
2. Peng G., K.L. Bailey, and M. Molloy. 2000. Field survey and preliminary evaluation of biological control of *Matricaria perforata* using fungal pathogens in Saskatchewan, Canada. Proc. 3rd International Weed Science Conference (Abstr.). Iguassu Falls, Brazil.
3. Peng G., K.L. Bailey, and K.N. Byer. 2000. Exploration of biological control of scentless chamomile (*Matricaria perforata*) using plant pathogens (Poster). Expert Committee on Weeds, 2000, Banff, Alberta.

Presentations

4. Presentation at Saskatchewan Scentless Chamomile Working Group Annual Meeting: "Potential for biological control of scentless chamomile using indigenous plant pathogens".
5. Presentation at USDA annual bioherbicide meeting: "Evaluation of plant pathogens for biological control of scentless chamomile".
6. Research progress update at Saskatchewan Scentless Chamomile Working Group Meeting by Dr. K.L. Bailey, April 2001.
7. Presentation at CABI Biosciences, Switzerland entitled "Utilization of plant fungal pathogens for biological control of scentless chamomile in western Canada". May 2001.
8. Presentation at Saskatchewan Scentless Chamomile Working Group Meeting entitled "Biological control of scentless chamomile using indigenous and exotic fungal pathogens". October, 2001.
9. Presentation at the annual meeting of the Saskatchewan group of Canadian Phyopathology Society. November, 2001. By Byer, K.N., Peng, G., Wolf, T.M. and Caldwell, B: "Application parameters - the potential impact on the efficacy of microbial weed control".

Extension publications

"Potential to use microbes to control scentless chamomile in Saskatchewan." In: Saskatchewan Alfalfa Seed Producers Association newsletter, October 2001.

Personnel

1. **Dr. Peng G.** Research Scientist, AAFC Saskatoon Research Centre (0.1 PY). Project Leader.
2. **Dr. Bailey, KL.** Research Scientist, AAFC Saskatoon (0.05 PY). Co-investigator.
3. **Kelly Byer.** Technician. AAFC Saskatoon (0.05 PY). Assisting in trial implementation and data analyses.
4. **Dr. Knud Mortensen**, MRJ Biosurveys, Balgonie, SK. A collaborative agreement was signed between AAFC and the company. Under the agreement, MRJ Biosurveys conducted surveys and field collections primarily in southern part of the province. Disease samples were send to AAFC Saskatoon Research Centre for pathogen isolation and biocontrol assessment.
5. **Sharon Leung:** part-time technician (0.5 PY) hired between April 1, 2000 and March 31, 2002 to assist field survey, pathogen isolation, and evaluation of biocontrol.
6. **Mark Taylor:** contractual research assistant (3 months) to assist efficacy evaluation on whole plants of scentless chamomile.
7. **Lane Gelhorn:** contractual research assistant (3 months) to assist evaluation of crop safety for efficacious fungal organisms to eliminate crop pathogens from further evaluation process.

8. **Wendy Guo:** contractual research assistant (3 months) to prepare plant materials and pathogen inoculum for evaluation trials.
9. **Faye Dokken**: University student and worked as a part time contractor (1 month) to prepare plant materials and microorganisms for efficacy evaluation.

Equipment

No equipment over $500 was purchased using the ADF grant fund.

Project Developed Materials:

Several promising fungal isolates have been identified as potential candidates for biocontrol of scentless chamomile. Their feasibility for use under Saskatchewan environment will be further evaluated including development of effective formulations and tank mixing strategy with chemical herbicides.

Project Photos

Nine illustration photos are included in Appendix 2 of this report.

Acknowledgement

This project is financially supported with funds from several organizations. Monetary contributions by Saskatchewan ADF of Saskatchewan Department of Agriculture and Food, Matching Investment Incentive of AAFC, and Philom Bios Inc. are gratefully acknowledged. In-kind contributions were made by MRJ Biosurveys, which allowed field surveys to cover a large territory across areas of weed infestation.

Expense Statement

A financial statement is included in the following page. A deficit of $12.5200 has occurred in anticipation that the amount will be reimbursed by ADF following the final report of project. Higher than planned expenditures were incurred in salaries due to a large number of samples collected from field surveys. Four casual technical assistants had to be hired at different times to alleviate backlog of sample processing and pathogen screening. However, overall expenditures stayed within the budget mainly because of additional financial subsidies from MII of AAFC on field surveys, travels, materials, and supplies.

Research Photos

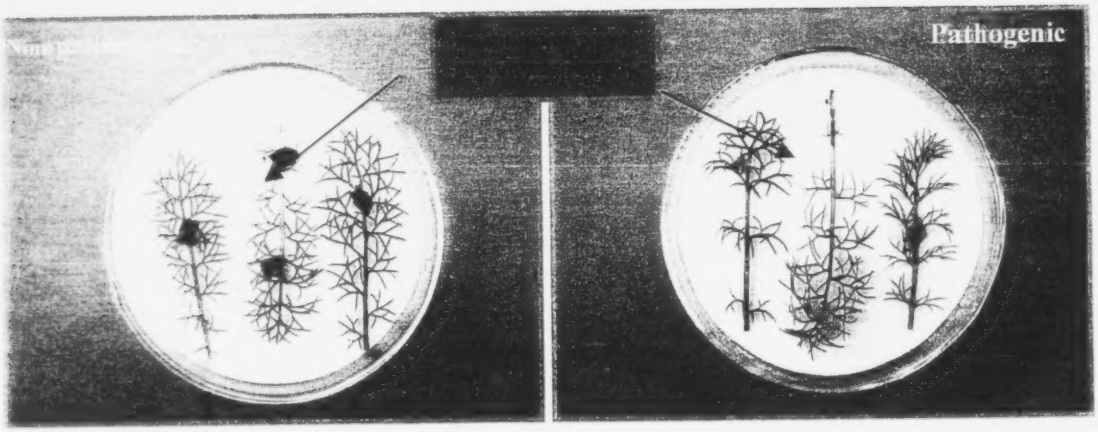


Photo 1. Bioassay. The organisms in the left and right plates are considered as nonpathogen and pathogen, repectively, based on the presence of disease symptoms originated from the inoculation using mycelium plugs.

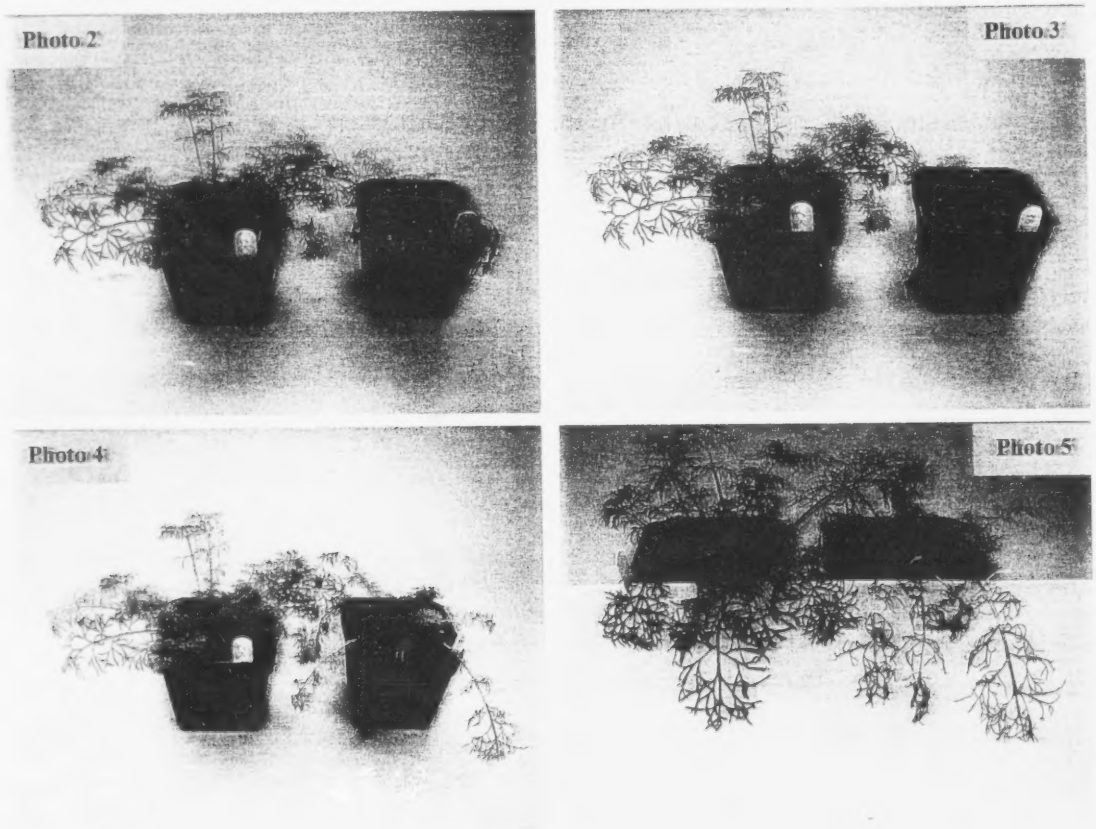


Photo 2 - 5. Disease caused by foliar inoculation with spore suspensions of 00-3B1 (Photo 2), 00-3C1 (Photo 3), and 00-2B3 (Photo 4), respectively, at approximately 7-leaf stage of scentless chamomile. Photo 5 shows close-up symptoms caused by 00-2B3.

Research Photos

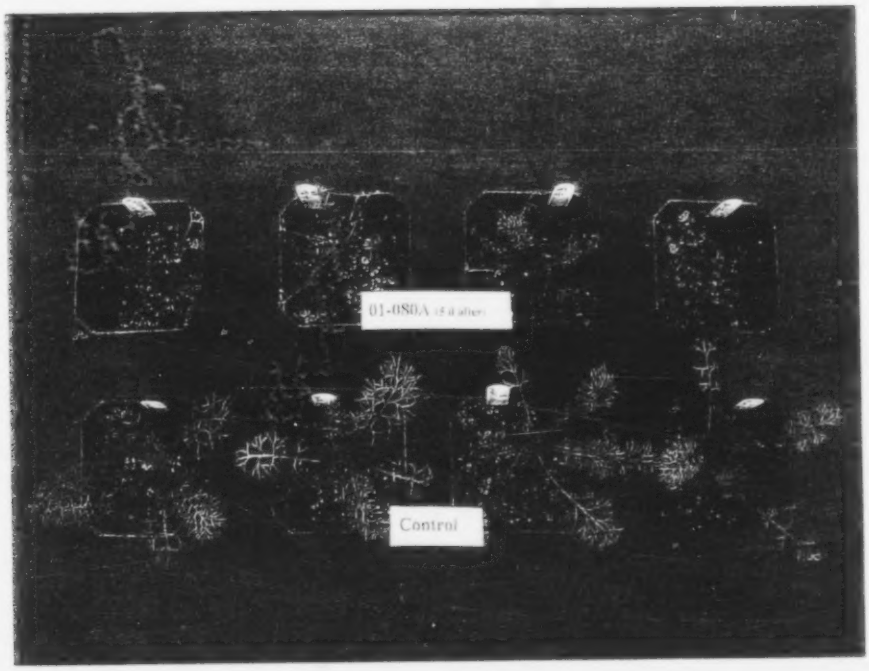


Photo 6. In the greenhouse, the fungal agent 01-080A controlled scentless chamomile at the 7-leaf stage effectively 5 days after the treatment.

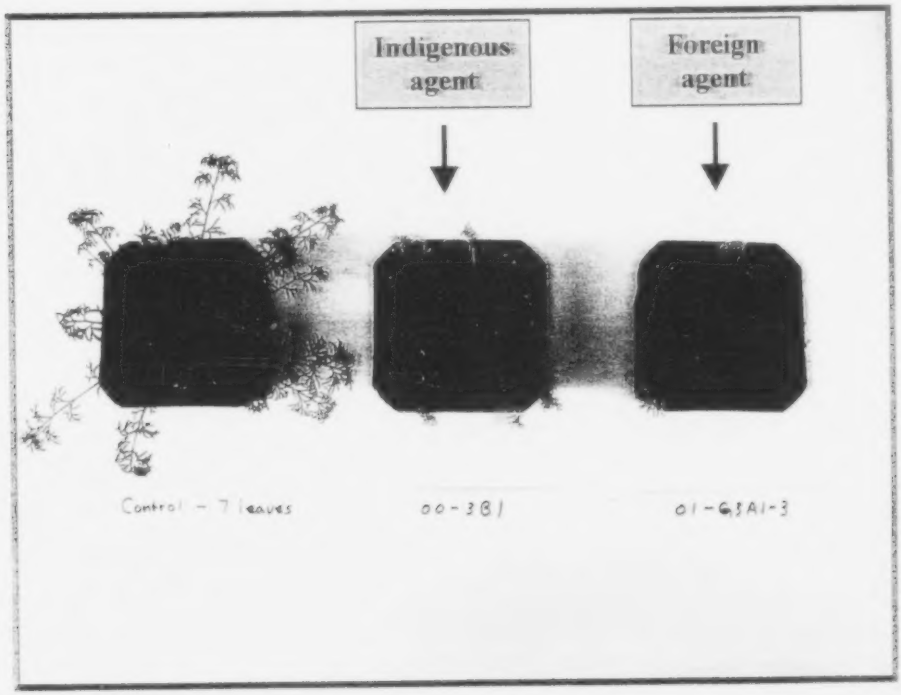


Photo 7. Comparison of weed control efficacy between indigenous agent 00-003B1 and a foreign strain of similar pathogen collected in Europe.

Research Photos

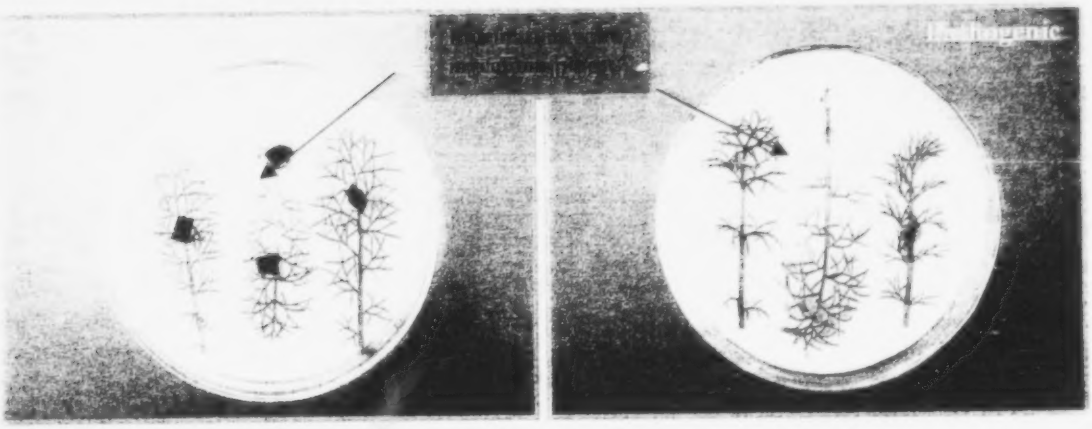


Photo 1. Bioassay. The organisms in the left and right plates are considered as nonpathogen and pathogen, repectively, based on the presence of disease symptoms originated from the inoculation using mycelium plugs.

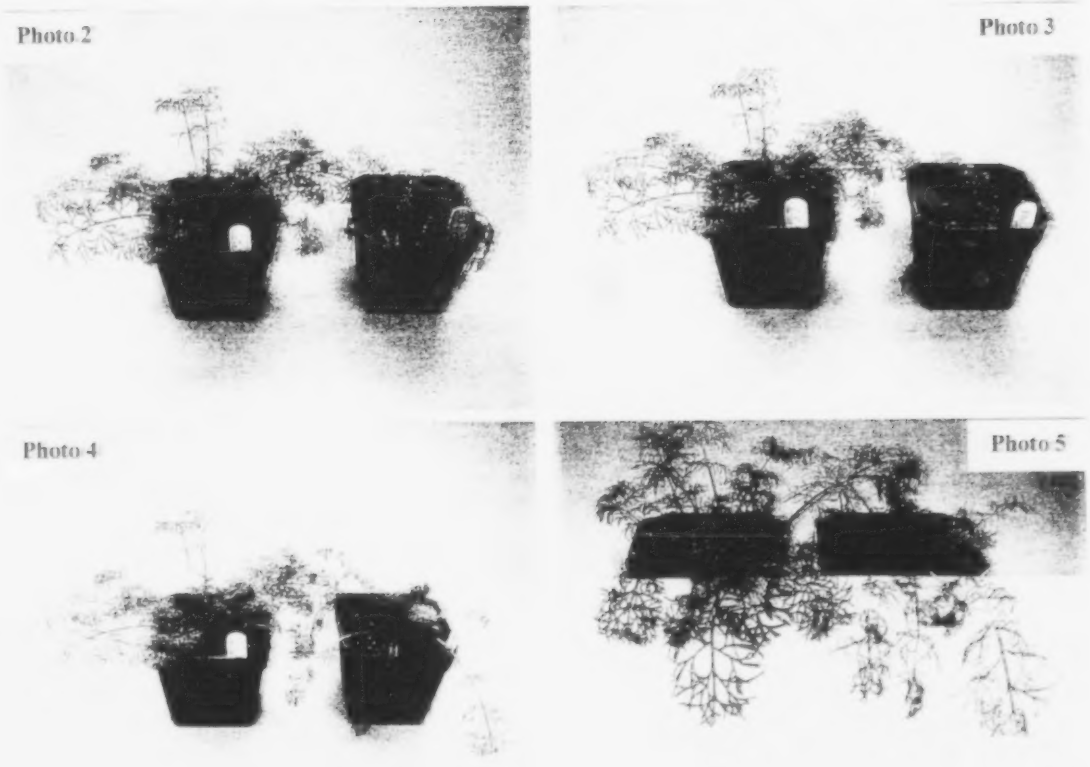


Photo 2 - 5. Disease caused by foliar inoculation with spore suspensions of 00-3B1 (Photo 2), 00-3C1 (Photo 3), and 00-2B3 (Photo 4), respectively, at approximately 7-leaf stage of scentless chamomile. Photo 5 shows close-up symptoms caused by 00-2B3.

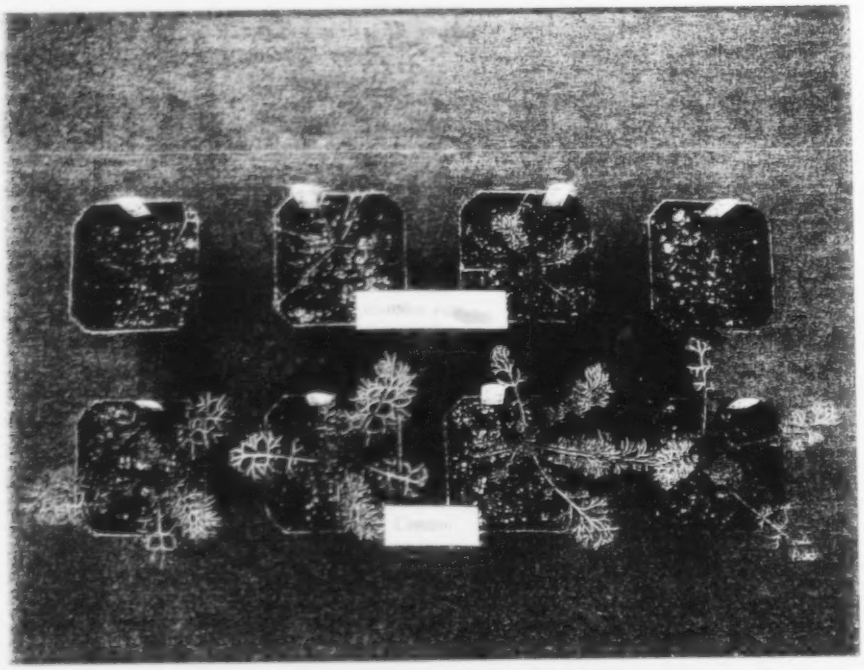


Photo 6. In the greenhouse, the fungal agent 01-080A controlled scentless chamomile at the 7-leaf stage effectively 5 days after the treatment.

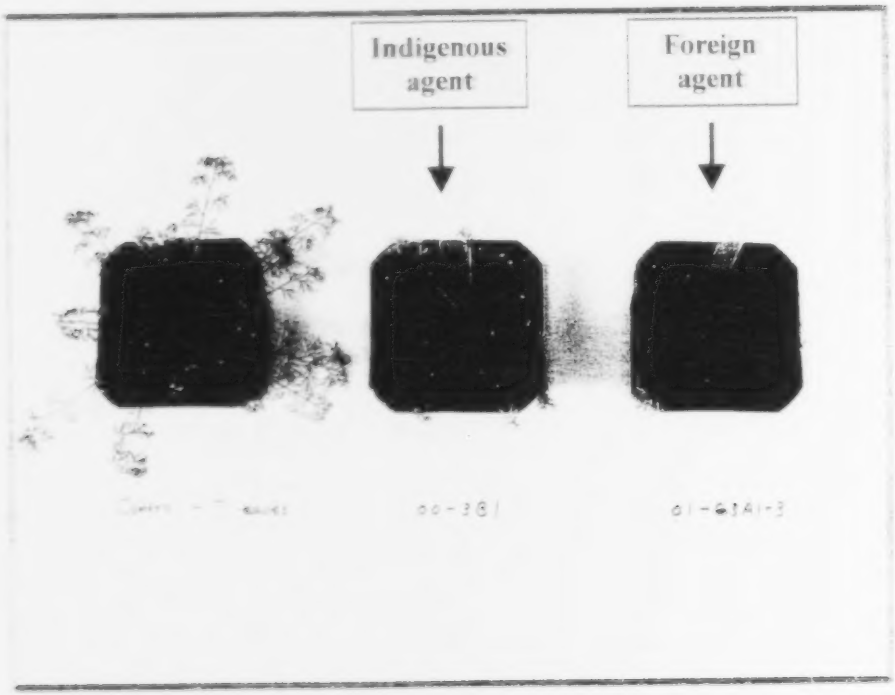


Photo 7. Comparison of weed control efficacy between indigenous agent 00-003B1 and a foreign strain of similar pathogen collected in Europe.

Research Photos

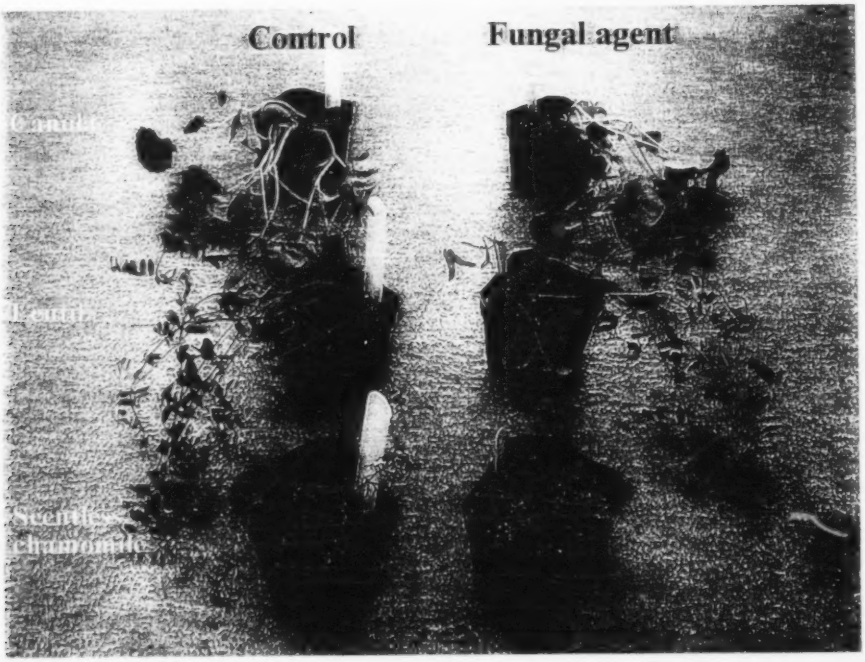


Photo 8. The fungal agent did not cause disease on any of the 14 species of prairie crops and native plants tested. The plants in the picture are merely examples.

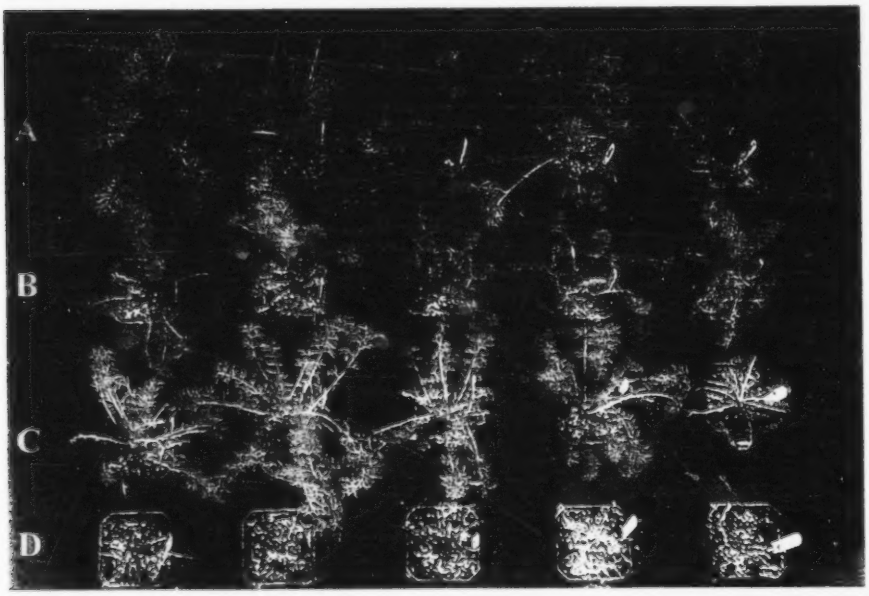


Photo 9. Synergy of a fungal agent with herbicide Sencor. Treatments from top to bottom:

A – Untreated control
B – Fungal agent alone
C – Sencor alone
D – Sencor plus fungal agent

Appendix 1. Fungal pathogens recorded in association with *Matricaria perforata* and closely related weed species.

Name	Host plants recorded/Host range	Symptoms	Sources
Albugo tragopogonis Pers. Ex S.F. Gray	Generally on: *Tragopogon* , but also *Chamomilla suaveolens, Antennaria, Artemisia, Centaurea, Helianthus, Senecio,* etc.	"White rust", causing white blisters	Farr *et al.* 1989, Ellis and Ellis 1987, Brandenburger 1985, Horst 1979
Alternaria alternata (Fr.) Kreissler	Plurivorous, Opportunistic	Necrotic spots on leaves and stems	Isolated from *M. perforata* in 1994 and '97 (diseased seedlings)
Ascochyta matricariae Grove	The species can be host specific	Leaf necrosis; can be quite damaging	Isolated from *M. perforata* 1994
Botrytis cinerea Fr.	Plurivorous, common grey mould	Leaf spot, fruit rot, petal specks	Isolated from *M. perforata* in 1993; Kay 1994
Cladosporium sp.	Ubiquitous and mostly saprophytic	Associated with leaf spots, necrosis, and lesions in plant tissues	Isolated from seedlings of *M. perforata* in 1997
Colletotrichum gloeosporioides (Penz.) Penz & Sacc.	Host-specific forms exist	Usually anthracnose with black and slightly sunken lesions.	Isolated from *M. perforata* in 1996
Entyloma matricariae Rostr. = *E. matricariae* Trail = *E. trailii* Mass.	According to Buhr (1964) only on *M. perforata.*	White smut, typically leaf chlorosis and necrosis	Ellis and Ellis 1987, Hegi 1987,Brandenburger 1985, Buhr 1964, Lindeberg 1959; IMI ref. no. 329906
Erysiphe cichoracearum	plurivorous; among many others on: *Chamomilla* spp. and *Achillea* spp.	Powdery mildew	Farr *et al.* 1989, Ellis and Ellis 1987, Brandenburger 1985, Horst 1979

..... Continues

Erysiphe polyphaga Hammarlund	plurivorous [*Oidium* is the anamorphic state]	Powdery mildew	Blumer 1967, Brandenburger 1985
Fusarium oxysporum Schlecht.	Can be host-specific, forma specialis known	Vesicular infections, distorted leaves/stems	Isolated from *M. perforata* in 1994
Gliocladium roseum Bainier	Mycoparasite	No details	Isolated from *M. perforata* in 1994
Helotium sp.	*Matricaria maritima*	No details	IMI record *
Microdochium sp.	Cereals	No details	Isolated from *M. perforata* in 1999
Monographella nivalis (Schaffn. E. Müll.)	Mainly known from cereal crops	No details	Isolated from *M. perforata* in 1996
Mycosphaerella sp.	*Matricaria maritima*	Leaf spot	IMI record
Oidium sp.	Plurivorous	Powdery mildew	Brandenburger 1985, Horst 1979, Blumer 1967
Olpidium brassicae (Woronin) P.A. Dang.	Plurivorous; besides many others, *Chamomilla suaveolens*	Disease on seedlings	Ginns 1986
Peronospora leptosperma De Bary	*Matricaria* spp., *Chamomilla* spp., *Anthemis* spp., *Tanacetum* spp.	Downy mildew	Ellis and Ellis 1987, Hegi 1987, Brandenburger 1985, Buhr 1964, Docters van Leeuwen 1957, Frank 1880; IMI ref. No. 353388
Peronospora radii De Bary = *P. danica* Gäum.	*Matricaria* spp., *Chamomilla* spp., *Achillea* spp., *Anthemis* spp., *Chrysanthemum leucanthemum, C. segetum*	Downy mildew; deformed ray and disc florets	Hegi 1987, Brandenburger 1985, Buhr 1964, Butler & Jones 1949, Frank 1880, IMI record
Phoma sp.	Plurivorous	Necrotic leaf spot	Isolated from *M. perforata* in 1993 and 2001; IMI record
Phomopsis albicans Roberge ex Desm	*M. perforata, Hypochaeris*	Necrotic stem lesions	Grove 1935, Ellis & Ellis 1987, isolated from *M. perforata* in 2001

.....Continues

Pleospora herbarum (Pers.)	*Matricaria maritime, Chamomilla recutita*	Necrotic stem lesions	Hegi 1987, IMI record, isolated in 2001 as *Stemphylium* sp.
Protomyces matricariae Syd.	*M. perforata*	Pale green, producing calluses on leaves	Buhr 1964, Brandenburger 1985
Puccinia matricariae Sydow = *P. tanaceti* ?	*Chamomilla recutita*	Rust	Brandenburger 1985, Gäumann 1959, IMI record
Ramularia matricariae Antokol ex W. Vassilijevsky & Karak = *Didymaria matricariae*	*Matricaria* spp.	White leaf spots/lesions;	Isolated from *M. perforata* in 1999, 2000; IMI ref. No's 7550 and 7551
Septoria matricariae Sydow	*Matricaria perforata, Chamomilla* spp., *Anthemis cotula*	Leaf spot	Ellis & Ellis 1987, Brandenburger 1985; IMI ref. No. 353746
Sphaerotheca erigerontis-canadensis	*Chamomilla suaveolens, Erigeron canadensis,*	Powdery mildew;	Ellis and Ellis 1987
Sphaerotheca fuliginea	*Chamomilla recutita, C. suaveolens, Veronica chamaedrys, Caltha*	Powdery mildew	Farr *et al.* 1989, Ellis and Ellis 1987, Ginns 1986, Brandenburger 1985; IMI record
Sphaerotheca humuli (D.C.) Burr. *var. fuliginea* (Schlecht.) Salm.	*Chamomilla suaveolens*	Powdery mildew	Horst 1979
Sphaerotheca macularis	Plurivorous; besides others on: *Chamomilla suaveolens and Anthemis cotula*	Powdry mildew;	Farr *et al.* 1989, Ellis and Ellis 1987, Horst 1979
Sphaerotheca xanthii	*Chamomilla suaveolens*	Powdery mildew;	IMI record

* CABI Bioscience, 2001: Database of fungal names (Funindex): http://194.131.255.3/cabipages/Names/Names.asp

Appendix 2. Description of collection sites for diseased scentless chamomile in Saskatchewan

Year	Site #	Location	Collector	Weed Density (plants/m^2)	Habitat	Weed Stage	Symptoms
1999	19	SE4-45-10W3 Rm 435	G. Peng-M.Molloy	26-50	Roadside	Seedling, seed setting	Stem lesions
1999	21	Gps 52-52.292NW107-26.044	G. Peng-M. Molloy	1-10	Slough edge	Seedling, flowering, seed setting	Stem lesions
1999	28	NE20-45-10W3 RM435	G. Peng -M. Molloy	1-10	Field crop -wheat	Vegetative	Leaf spot-stem lesion
1999	29	NE29-44-10W3 RM435 N of Hafford	G. Peng	>50	Field crop-alfalfa	Flowering , seed setting	Leaf spot-stem lesion
2000	1	NE29-44-10W3 Rm435	M. Molloy-S. Leung	1-10	Field crop-alfalfa	Vegative	Stem lesions
2000	2	SE4-44-10W3	M. Molloy-S. Leung	1-10	Roadside	Seedling vegetive	Stem lesions top dieback
2000	3	NE27-44-10W3 RM435	M. Molloy-S Leung	11-25	Field crop stubble wheat	Vegetative	Stem lesions
2000	4	SW4-44-10 W3 RM 435	M. Molloy-S Leung	1-10	Field crop – stubble wheat	Vegetative	Stem lesions
2000	5	NW8-45-9W3 RM435	M. Molloy-S. Leung	1-10	Field crop	Vegetative	Stem lesions
2000	6	SE15-45-9 RM 435	M. Molloy-S. Leung	>50	Field crop-wheat	Seedling, vegetative	Leaf spots
2000	7	NE17-44-9W3 RM435	M. Molloy-S.Leung	1-10	Field crop-wheat stubble	Vegetative	Stem lesion
2000	8	SE17-44-9W3 RM435	M. Molloy-SLeung	26-50	Field crop-stubble wheat	Vegetative	Stem lesion
2000	9	SE 33-43-10 RM435	M.Molloy-S.Leung	1-10	Field crop – stubble wheat	Vegetative	Leaf spots
2000	10	NW3-44-10W3 RM435	M.Molloy-S.Leung	11-25	Field crop, roadside	Vegetative	Leaf spots
2000	11	NE17-44-10W3 RM435	M. Molloy-S. Leung	11-25	Roadside	Vevetative	Leaf spot
2000	12	NE19-44-10 W3 RM435	M.Molloy-S.Leung	1-10	Field crop	Vegetative	Leaf spot

2000	15	SW10-45-12 W3 RM 436	M. Molloy & S. Leung	1-10	Field crop	Vegetative	Leaf spots, Stem lesions
2000	17	NE27-44-12 W3 RM436	M.Molloy-S.Leung			Vegetative, bolting	
2000	18	SE23-44-12 W3 RM436	M.Molloy-S.Leung	1-10	Field crop	Vegetative	Leaf spot
2000	19	SW 27-44-11-W3 RM446	M.Molloy-S.Leung	1-10	Roadside	Vegetative, bolting	Leaf spots
2000	20	NE14-44-11 W3 RM436	M.Molloy-S.Leung	1-10	Field crop-wheat	Seedling bolting	Leaf spots stem lesions
2000	21	SE24-43-11 W3 RM 436	M.Molloy-S.Leung	11-25	Field crop-wheat stubble	Bolting	Leaf spots
2000	26	SE3-27-29 W2 RN252 behind HUSKY gas station	M.Molloy-S.Leung	1-10	Roadside	Bolting	Leaf spot
2000	30	Regina - 3900 block Vic Ave RM159	M.Molloy	1-10	Back alley	Bolting	Leaf spots
2000	32	NW2-42-26 W2 RM401	M.Molloy-S.Leung	11-25	Field crop -wheat	Bolting, flowering	Leaf spot, top dieback
2000	34	SE34-42-26 W2 RM401	M. Molloy-SLeung	1-10	Field crop -wheat	Bolting	Stem lesions top dieback
2000	37	SW&7-42-25-W2 RM 401	M.Molloy-S.Leung	1-10	Roadside	Vegetative	Leaf spots
2000	42	NW7-36-17 W2 RM338	M. Molloy-F. Dokken	26-50	Roadside	Seedling, flowering	Leaf spots
2000	46	NE20-36-18W2 RM338	M. Molloy-F. Dokken	1-10	Roadside	Vegetative	Leaf spot
2000	49	SE30-34-19 W2 RM 339	M. Molloy-F. Dokken	1-10	Roadside	Vegetative	Leaf spots
2000	57	SW21-50-20 W2 RM491	M.Molloy-S.Leung	11-25	Roadside	Seedling, flowering	Leaf spot stem lesion,
2000	61	SW15-50-2W3 RM493	M.Molloy-S.Leung	1-10	Roadside	Vegetative, bolting	Leaf spot
2000	62	SW17-50-2 W3 RM493	M.Molloy-S.Leung	11-25	Roadside	Vegetative	Leaf spot

2000	66	SW20-51-3 W3 RM493	M. Molloy-SLeung	1-10	Field crop - pea	Vegetative	Leaf spot
2000	70	Saskatoon -near bus stop at Lawson Ht Mall RM344	M. Molloy	11-25	Other -curb	Flowering	Leaf spot, stem lesion
2000	71	NW 19-34-22 W3 RM 350	M. Molloy & S. Leung	1-10	Roadside-ditch	Flowering	Leaf spot, stem lesions, top dieback
2000	74	Nw 25-34-23 W3 RM 351	M. Molloy & S. Leung	1-10	Road side	Flowering	Leaf spot, stem lesions
2000	75	SE 6-36-25 W3	M. Molloy & S. Leung	1-10	Road side	seed setting	Leaf tip dieback
2000	76	NE7-36-25 W3 RM351	M. Molloy-SLeung	>50	Roadside	Flowering	Leaf spot top dieback
2000	79	NW16-36-25 W3 RM351	M. Molloy-SLeung	26-50	Field crop – summerfallow	Flowering	Leaf spot, stem lesion, top dieback
2000	91	SW1-19-17 W2 RM158	K. Mortensen	11-25	Other -summer fallow	Bolting	Stem lesion, top dieback
2000	92	SE 1-19-16W2 (3 N & 1W of McLean)	K. Mortensen	26-50	Field crop – slough edge	Bolting, flowering	Root decay, stem lesions, top dieback
2000	93	NE1-19-17 W2 Rm158	K. Mortensen	26-50	Other around slough edge	Seedling bolting	Wilting
2000	94	SE12-19-17 W2 RM158	K. Mortensen	26-50	Field crop - pea	Bolting, flowering	Chlorosis
2000	95	SW18-20-17 W2 RM158	K. Mortensen	26-50	Field crop – fallow field	Bolting, flowering	Stem lesions, top dieback
2000	96	NE8-20-18 W2 RM158	K. Mortensen	>50	Field crop-summer fallow	Seedling, bolting	Wilting
2000	98	SW11-27-29 W2 RM252	M.Molloy-S.Leung	1-10	Roadside	Flowering	Stem lesion, top dieback
2000	106	SE17-18-20 W2 RM159	K. Mortensen	>50	Roadside	Flowering	Stem lesion, top dieback
2000	107	SW 25-18-20-W2	K. Mortensen	>50	Roadside - creek edge	Flowering	Stem lesions, top dieback, sclerotinia wilt
2000	108	SW11-16-24 W2 RM160	K. Mortensen	11-25	Roadside	Flowering	Stem lesion, top dieback

2000	109	NE 29-13-22-W2 (5 S & 3 W of Rouleau)	K. Mortensen	>50	Roadside - creek edge	Vegetative, flowering	Wilting, dark roots
2000	110	Shaunovan junction of HWY 13 & 37 RM 78	K. Mortensen	>50	Slough edge	Flowering, seed setting	Top dieback, wilting
2000	111	2-8-20 W2 RM79	K. Mortensen	>50	Roadside – railroad	Seedling, seed setting	Wilting
2000	112	Dollard 1-8-20 W2 RM 79	K. Mortensen	26-50	Roadside - Pool Elevator site	Flowering, seed setting	
2000	113	2 miles W Swift Current	K. Mortensen	>50	Roadside – railroad	Seedling, flowering	Top dieback, wilting
2000	114	2 miles W Swift Current	K. Mortensen	>50	Dugout	Seedling, flowering	Top dieback
2000	116	Chamberlain-Bennett Garage	Margaret Molloy		Other summer fallow	Flowering	Leaf spot
2000	116	Chamberlain	M. Molloy	1-10	Other summer fallow	Flowering	Leaf spot
2000	118	27-21-18W2 RM218	K. Mortensen	11-25	Roadside	Flowering	Top dieback
2000	118	27-21-18 W2 RM218	K. Mortensen	11-25	Roadside	Flowering	Top dieback, wilting
2000	119	30-26-10 W2 RM 246	K. Mortensen	26-50	Roadside - beside cereal crop	Bolting, flowering	Top dieback, wilting
2000	120	27-27-9 W2 RM245	K. Mortensen	11-25	Field crop – stubble (isolated)	Flowering	Aster yellows
2000	125	NW35-18-17 W2 RM158	K. Mortensen	>50	Summerfallow	Flowering	Wilting
2000	127	SW13-20-18 W2 RM158	K. Mortensen	1-10	Field crop - flax	Flowering	Stem lesions, rust
2000	129	NW 12-20-18 W2 RM 158	K. Mortensen	>50	Field crop – canola	Flowering	Stem lesions, wilting
2000	130	SW 15-20-18 W2 RM 158	K. Mortensen	11-25	Weedy stubble	Flowering	Wilting
2000	132	NE8-20-18 W2 RM 158	K. Mortensen	11-25	Weedy stubble	Flowering, seed setting	Stem lesions, top dieback

2000	140	NE 19-35-1 W3 RM 343	M. Molloy & S. Leung	1-10	Roadside	Flowering	Leaf spot
2000	150	3-30-34 W1 RM271	K. Mortensen	26-50	Field crop – canola	Flowering, setting seed	Wilting
2000	151	10-22-2-W2 (6 miles W & 4 miles S of Bredenbury) RM 213	K. Mortensen	11-25	Slough - dried out	seed setting	Wilting
2000	167	NW Lashburn RM472	G. Peng	1-10	Field crop	seed setting	Chlorosis
2000	171	Hwy3 section 10+11 2kmN of Hyw3	G. Peng	1-10	Roadside	Flowering, seed setting	Leaf spot
2000	173	N of hwy 3 RM502	G. Peng	11-25	Field crop	Flowering, setting seed	Leaf spot
2000	175	section 22&23 W of Hyw 3 2km S of river RM 502	G. Peng	1-10	Field crop	Seedling	Leaf spot
2000	178	Section 22 Nof Hyw3 2KmS of river	G.Peng	11-25	Field crop	Seedling	Stem lesion
2000	179	RM 502 Section 22, N of highway 3, 2 km S of river	Gary Peng	1-10	Roadside	Seedling	Stem lesion
2000	186	N 2Km of St. Walburg	G. Peng	1-10	Field crop – barley		Leaf spot
2000	190	4 km N of St Walburg RM501	G. Peng	1-10			Leaf spot
2000	193	3 km S of St Walburg RM501	G. Peng	1-10			Leaf spot
2000	213	N of Beatly RM429	G. Peng	11-25	Waste land	Seedling	Leaf spot
2000	215	N of Beatly RM 429	G. Peng	1-10	Waste land	Seedling	Leaf spot
2000	218	RM 429 (1 Km W of Melfort)	G. Peng	1-10	Roadside	Flowering	Leaf tip dieback
2000	219	Tisdale, TNP 45 Section 15 &16 RM427	G. Peng	1-10	Roadside	Seedling	Leaf spot
2000	221	NE of Tisdale Twp 45 section 15	G. Peng	11-25	Pasture	Flowering	Leaf spot

2000	225	3km S of Nipawin RM487	G. Peng	11-25		Flowering, seed setting	Leaf spot
2000	229	S of Garrick Hyw 55 RM488	G. Peng	1-10	Pasture	Flowering	Leaf spot
2000	246	14-22-28 RM290	G. Peng	>50	Field crop	Seedling	Leaf spot
2000	247	11-28-22W3 RM290	G. Peng	1-10	Roadside	Seed setting	Root rot
2000	248	14-22-28 RM290	G. Peng	>50	Field crop	Seedling	Leaf spot
2000	249	14-22-28 RM290	G. Peng	>50	Field crop	Seedling	Leaf spot
2000	250	Beaufield 19-22-31 RM320	G. Peng	11-25	Field crop	Seedling	Leaf spot
2000	252	19-31-22W3 RM321	G.Peng	26-50	Roadside	Flowering	Stem lesion
2000	258	2 Km S of Perdue RM 346	G. Peng	1-10	Roadside	Seed setting	Leaf spot
2000	262	Spiritwood RM 496	G. Peng	1-10	Roadside - Pool Elevator site	Seedling	Leaf spot
2000	264	7 Km N of Shellbrock RM 493	G. Peng	>50	Roadside	Seedling	Leaf spot
2001	4	Dallard S2-8-20W3	K Mortensen	26-50	Other	Seedling, vegetative	Wilting
2001	6	Swift Current S27-15-14W3	K. Mortensen	>50	Roadside	Vegetative	Wilting
2001	7	Hafford	G. Peng	1-10	Field crop	Vegetative, bolting	Leaf spot
2001	8	Hafford	G. Peng	1-10	Field crop	Vegetative	Leaf spot
2001	9	Hafford	G. Peng	26-50	Field crop	Vegetative	Stem lesion
2001	10	NW Hafford	G Peng	1-10	Field crop	Vegetative	Leaf spot

2001	11	Hafford Section 17	G. Peng	1-10	Field crop	Vegetative	Leaf spot
2001	12	Hafford section 7	G. Peng	1-10	Field crop	Vegetative	Leaf spot
2001	13	section 34 Rm 436	G. Peng	1-10	Field crop	Vegetative	Leaf spot
2001	18	Edenwold NE5-20-18W2	K. Mortensen	>50	Other summer fallow	Bolting	Wilting
2001	19	Fillmore SW18-11-10W2	K. Mortensen	11-25	Field crop alfalfa	Seedling	Wilting
2001	20	Brownkng SW31-5-5W2	K. Mortensen	1-10	Roadside	Bolting	Top dieback
2001	21	Lampman SE2-5-4W2	K Mortensen	26-50	Other stubble	Seedling, bolting	Wilting
2001	24	Mc Lean SE4-19-15W2	K Mortensen	11-25	Roadside	Flowering	Wilting
2001	25	Odessa SE31-14-13W2	K Mortensen	11-25	Field crop	Seedling	Stem lesion
2001	26	Carlyle NE20-7-2W2	K Mortensen	>50	Roadside	Seedling, vegetative, bolting	Stem lesion
2001	27	Alamada S10-4-3W2	K Mortensen	>50	Roadside	Bolting	Wilting
2001	28	Wolseley	K.Mortensen	>50	Roadside	Seedling, bolting, flowering	Wilting
2001	29	Welwyn SE2-16-30W1	K. Mortensen	1-10	Roadside	Flowering	Stem lesion
2001	30	Whitewood NW22-17-1W2	K. Mortensen	1-10	Roadside	Flowering	Stem lesion
2001	31	Whitewood E9-18-1W2	K. Mortensen	11-25	Roadside	Bolting, flowering	Stem lesion
2001	32	Whitewood Se36-17-2W2	K. Mortensen	26-50	Pasture hayfield	Seedling, bolting, flowering	Stem lesion, wilting
2001	34	Edenwold SW1-19-17W2	K. Mortensen	>50	Roadside slough edge	Seedling, flowering	Wilting

2001	35	Edenwold NE22-19-17W2	K. Mortensen	26-50	Roadside	Flowering	Wilting
2001	36	Balgonie SW15-18-17W2	K. Mortensen	>50	Roadside	Flowering	Wilting
2001	37	Balgonie SE15-18-17W2	K. Mortensen	>50	Roadside	Flowering	Wilting
2001	38	Grenfell SW17-17-6W2	K. Mortensen	11-25	Roadside	Flowering	Stem lesion
2001	39	Grenfell E 9-17-7W2	K. Mortensen	26-50	Roadside	Flowering	Stem lesion
2001	40	Grenfell S 6-17-6W2	K. Mortensen	>50	Field crop stubble	Seedling, flowering	Stem lesion
2001	41	Broadview NE17-17-5W2	K. Mortensen	26-50	Roadside	Vegetative, bolting	Stem lesion, wilting
2001	42	Broadview NE20-17-4W2	K. Mortensen	26-50	Roadside	Flowering	Wilting
2001	43	Browdview NW16-17-4W2	K. Mortensen	26-50	Field crop stubble	Seedling, flowering	Wilting
2001	44	Broadview NE9-17-4W2	K. Mortensen	26-50	Field crop stubble	Flowering	Chlorosis
2001	45	Broadveiw SW3-16-4W2	K. Mortensen	26-50	Field crop stubble	Flowering	Stem lesion
2001	52	N of Grenfell	G. Peng	11-25	Field crop	Vegetative, seed setting	Leaf spot
2001	52	NE of Broadview	G. Peng	1-10	Roadside	Flowering	Stem lesion
2001	53	N of Grenfell	G. Peng	1-10	Field crop	Vegetative	Leaf spot
2001	54	N of Grenfell	G. Peng	1-10	Field crop	Vegetative	Leaf spot
2001	55	N of Grenfell	G. Peng	1-10	Field crop		Stem lesion
2001	56	NE of Broadview RM 154	G Peng	1-10	Roadside	Flowering	Leaf spot

2001	58	N of Broadview	G. Peng	>50	2field crop	Seedling	Leaf spot
2001	59	N of Broadview	G. Peng	>50	Other fallow	Vegetative	Leaf spot
2001	60	NE of Broadview	G. Peng	26-50	Field crop	Seedling	Leaf spot
2001	61	NEof Broadview	G. Peng	26-50	Field crop	Vegetitive	Leaf spot
2001	62	3m NE of Whitewood RM153	G. Peng	1-10	Roadside	Vegetative	Leaf spot
2001	64	NE of Whitewood	G. Peng	1-10	Roadside	Seedling	Leaf spot
2001	65	4m NE of Broadview	G. Peng	1-10	Field crop	Vegetative	Leaf spot
2001	66	1/2m N of Moosemin RM121	G. Peng	1-10	Roadside	Seedling	Leaf spot
2001	67	1/2m N of Moosemin RM121	G. Peng	1-10	Roadside	Seedling	Leaf spot, stem lesion
2001	74	5m E Willmar RM33	G. Peng	>50	Roadside	Seedling	Leaf spot, stem lesion
2001	75	2m S off Carlye RM63	G. Peng	11-25	Roadside	Seedling	Leaf spot
2001	76	1m SE of Kipling RM94	G. Peng	26-50	Field crop	Seedling	Leaf spot
2001	79	1/2m NW Kipling	G. Peng	1-10	Roadside	Seedling	Leaf spot
2001	80	1/2 m W Kipling	G. Peng	1-10	Roadside	Seedling	Leaf spot
2001	81	4m N 1m E Hafford	K. Byer	1-10	Roadside	Vegetative	Stem lesion, top dieback
2001	82	4m NE Hafford	K. Byer	1-10	Roadside	Seedling	Stem lesion, top dieback

Appendix 3: Detailed results for individual studies with statistical analyses.

I. Weed-control efficacy for pathogenic isolates at whole-plant level.

Isolate	% Disease		% Fresh Weight Reduction
	Week 1	Week 2	
00-001A2A	0.00	0.00	11.33
00-001C2	0.00	0.00	11.28
00-002B1	0.00	0.00	16.85
00-002B3	29.38	44.38	61.32
00-003B1	68.75	68.75	84.68
00-003B3	0.63	0.00	1.28
00-003C1	32.50	32.50	50.34
00-003C3	0.00	0.00	21.01
00-003C4	20.63	20.63	36.50
00-003D1	11.88	11.88	34.25
00-004A	0.00	0.00	17.16
00-004B2	0.00	0.00	23.13
00-004B3	1.25	1.25	-12.06
00-004B5	0.63	0.63	-29.62
00-004C1E	0.63	0.63	-4.57
00-004C2B	0.63	4.38	-11.56
00-004C2C	2.50	1.25	-24.76
00-005C1	0.00	0.00	-15.09
00-005C2	0.00	0.00	-19.41
00-005E1	0.00	0.63	-18.33
00-005E2	0.00	0.63	-30.19
00-006A2	0.00	0.00	10.61
00-006B1	1.25	0.00	-34.37
00-006C1	0.00	0.00	-20.96
00-006D	0.63	0.00	15.84
00-007	4.38	1.25	-10.05
00-008C	0.00	0.00	-12.01
00-009B	1.25	1.88	39.18
00-010A2	0.63	0.00	-3.71
00-010C1	0.00	0.00	3.06
00-012A1	7.50	0.63	27.81
00-012B1	3.75	0.63	26.37
00-012B3	5.00	1.25	33.44
00-015B1	5.00	0.63	8.84
00-015B5	0.00	0.63	1.39
00-015C2	0.63	0.00	18.33
00-017A	0.00	0.00	21.54
00-017B1	0.00	0.00	0.28
00-017B2	0.00	0.00	-25.00
00-017C1	0.00	1.25	32.78
00-017C2	0.00	0.00	8.96
00-017C3	0.00	0.63	23.49
00-018C1	0.00	0.00	14.89
00-018C2	0.00	0.00	2.59
00-018C3	0.00	0.00	-16.98
00-018C5	0.00	0.00	8.74

Isolate	% Disease		% Fresh Weight Reduction
	Week 1	Week 2	
00-019A2	0.00	0.00	32.46
00-020A2	0.00	0.00	16.56
00-020A3	0.00	0.00	-27.63
00-020B2	0.00	1.25	21.91
00-021A	13.13	5.00	35.53
00-021B	0.00	0.00	16.42
00-021C1	0.00	0.00	0.16
00-021C3	0.00	0.00	10.07
00-021C4	0.00	0.00	2.24
00-026A1	4.38	5.00	14.21
00-026A2	11.88	1.88	36.21
00-026B	0.63	0.63	31.03
00-030B3	0.00	0.00	21.32
00-030B4	0.63	0.63	14.93
00-031A1	0.63	0.00	8.58
00-031B2	3.75	3.75	-1.32
00-032A	0.00	0.00	10.59
00-032D	4.38	0.63	-40.35
00-034A2	1.25	0.00	-26.32
00-034B	0.00	1.88	32.43
00-034D1	0.00	0.00	-16.23
00-040A1	0.00	0.00	6.58
00-040A2	1.25	0.00	-15.35
00-042A1	0.00	0.00	-24.56
00-042A2	3.75	0.63	35.53
00-042D1	0.00	0.00	-33.77
00-042D2	4.38	1.25	9.68
00-044A	0.00	0.00	-0.97
00-045	0.00	0.00	15.52
00-047A1	0.00	0.00	-19.95
00-047A2	0.63	0.00	18.97
00-047A3	0.00	1.25	18.77
00-049	0.00	0.00	-19.30
00-050A	4.38	0.63	13.06
00-050B1	0.00	0.00	18.02
00-050B2	0.00	0.00	2.27
00-054A1	0.00	0.00	21.17
00-054A2	4.38	4.38	44.59
00-054B	0.00	0.00	16.50
00-055A	0.63	1.25	13.51
00-055B	0.00	0.63	25.89
00-057A	0.63	0.00	16.89
00-057B	0.63	0.00	25.86
00-057C	0.00	0.00	20.69
00-062A3	0.00	0.00	29.31
00-062C1	1.25	1.25	27.70

Isolate	% Disease		% Fresh Weight Reduction
	Week 1	Week 2	
00-063A2	1.25	0.63	11.26
00-063C1	0.00	0.00	17.24
00-063C2	32.50	26.88	46.40
00-063C3	1.25	0.63	24.77
00-066A1	1.25	0.00	32.76
00-066A2	0.63	0.00	29.31
00-066A3	0.00	0.00	22.41
00-068A1	0.00	0.00	17.46
00-079A2	0.00	0.00	16.62
00-079C	0.00	0.00	-28.30
00-079E	87.50	87.50	95.49
00-091B	0.00	0.00	-7.55
00-093A	0.00	1.25	20.00
00-093C	0.63	0.63	43.10
00-093D	0.00	0.00	14.93
00-093E	0.00	0.00	5.35
00-094B2	0.00	0.00	24.51
00-095C1	1.25	11.88	34.08
00-096B2	0.00	1.25	-15.09
00-098B2	51.25	45.00	67.04
00-098C2	87.50	87.50	100.00
00-106A1	0.63	0.63	19.32
00-106B	0.00	0.00	25.17
00-106C	0.00	0.00	52.81
00-108A	0.63	3.75	4.63
00-108B1	0.00	0.00	14.37
00-108B2	0.63	1.88	13.43
00-108C	5.63	14.38	32.51
00-111B	68.75	56.25	74.65
00-113A	4.38	7.50	-6.24
00-114A	0.63	0.63	12.68
00-114E	5.63	17.50	37.46
00-118B	0.00	0.00	21.12
00-120B	1.25	1.88	19.08
00-125D	0.00	0.00	7.28
00-127A1	0.00	0.00	27.98
00-127C1	0.00	0.00	-5.58
00-132A2	0.00	0.00	-6.95
00-132C	0.00	0.63	0.97
00-150A1	1.25	0.63	40.73
00-167A1	0.00	0.00	-11.17
00-167B	0.63	0.00	16.75
00-173C1	0.00	0.63	48.74
00-173C2	0.00	0.00	18.69
00-190A2	8.13	11.88	50.48
00-190D2	0.00	0.00	7.04
00-193A	0.00	1.88	39.46
00-193C	62.50	68.75	83.11
00-213A1	0.00	0.00	-7.77
00-213B	0.00	1.25	12.26

Isolate	% Disease		% Fresh Weight Reduction
	Week 1	Week 2	
00-215A	1.25	5.63	32.69
00-215B	14.17	8.75	26.45
00-225A2	0.63	1.25	-30.99
00-229A	0.00	0.00	30.04
00-229B	0.63	0.63	8.05
00-229C	0.00	0.63	-26.36
00-246A	26.25	15.00	22.11
00-246B	0.00	0.63	3.53
00-248A1	27.92	15.00	37.59
00-248A2	0.00	0.00	-19.11
00-248B	0.00	0.00	15.19
00-248C	0.63	0.63	18.73
00-249B	0.00	0.63	40.28
00-258	0.00	0.00	11.17
00-262B	8.13	13.75	55.60
00-264A1B	1.25	3.75	26.40
00-264A1C	0.63	0.63	25.38
00-264A2A	0.00	0.63	35.82
00-264A2B	0.00	0.63	25.91
00-264A2C	1.25	8.13	24.87
00-264B1A	0.00	1.25	19.68
00-264B1B	0.00	0.00	2.54
00-264B2C	0.00	0.00	8.58
01-004A2	3.75	3.75	19.75
01-004A3	0.00	0.00	-10.76
01-004A4	8.75	5.63	-14.31
01-004A5	5.63	5.63	-43.56
01-005B	25.00	2.50	2.00
01-006A2	13.75	5.00	2.25
01-007A2	0.00	0.00	-3.14
01-008A3	9.38	2.50	19.33
01-008A5	0.00	0.00	-38.57
01-009A	0.00	0.00	2.67
01-010A3	0.00	0.00	-37.22
01-010A4	0.00	0.00	-23.77
01-012A1	13.75	2.50	21.33
01-013A1	0.00	0.00	-8.67
01-013A2	9.38	2.50	34.00
01-013A3	2.50	0.00	-36.00
01-013A4	1.25	0.00	-7.33
01-013A5	0.63	1.25	-36.88
01-018A1	0.00	1.25	-34.98
01-019A	1.25	1.25	5.53
01-019B	0.63	1.88	11.69
01-020	38.13	26.25	26.71
01-021A	29.38	9.38	30.67
01-021B	5.00	5.00	-52.70
01-021D	0.63	0.63	-9.21
01-025A1	1.25	0.00	-55.80
01-028B1	8.13	1.88	-28.46

Isolate	% Disease		% Fresh Weight Reduction
	Week 1	Week 2	
01-028B2	8.75	5.63	-48.49
01-031A1	0.00	0.00	-32.29
01-034A3	5.00	8.75	11.67
01-034B2	0.00	5.63	2.41
01-035B2	13.75	3.75	17.43
01-037B1	1.25	12.50	25.50
01-037B2	1.88	1.88	34.77
01-038A1	8.75	5.63	28.75
01-038A2	5.00	5.63	18.67
01-039A	2.50	0.00	10.46
01-039B1	0.00	0.00	-17.04
01-041A1	7.50	2.50	6.02
01-041A2	5.63	2.50	21.50
01-041A4	0.00	0.00	-11.26
01-042C	13.75	3.75	22.52
01-043A2	8.75	8.13	31.70
01-045A2	0.00	0.00	-7.77
01-052A21	21.38	38.38	45.75
01-054A	36.25	29.38	35.80
01-055	18.13	13.75	28.40
01-061	29.38	13.75	11.73
01-062A	8.13	9.38	16.67
01-065A3	0.00	0.00	10.80
01-076A2	9.75	50.00	60.80
01-080A1	75.00	75.00	77.78
99-021A1	0.00	0.83	29.60

II. Initial host-range studies

Isolate	Observation	Treatment	Scentless Chamomile	Chickpea	Flax	Lentil	Polish Canola	Wheat
00-002B3	% Disease: Week 1	Inoculated	6.67	5.00	5.83	2.50	13.33	0.83
		Control	0.00	0.00	0.00	10.00	0.00	0.83
	% Disease: Week 2	Inoculated	6.67	5.00	5.00	1.67	1.67	0.00
		Control	0.00	0.83	0.00	1.67	5.00	0.00
	% Fresh Weight Reduction	Inoculated	20.52	-12.84	26.69	0.25	1.78	-14.03
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-003B1	% Disease: Week 1	Inoculated	10.83	1.67	6.67	2.50	0.83	2.50
		Control	0.00	1.67	10.83	2.50	12.50	0.00
	% Disease: Week 2	Inoculated	2.50	2.50	6.67	10.83	2.50	0.00
		Control	0.00	5.83	18.33	15.00	13.33	0.00
	% Fresh Weight Reduction	Inoculated	40.30	-11.72	-45.20	-28.73	-12.32	1.25
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-003C1	% Disease: Week 1	Inoculated	37.50	0.00	26.67	5.83	0.00	22.50
		Control	0.00	0.00	5.83	5.83	0.00	15.00
	% Disease: Week 2	Inoculated	18.33	0.00	13.33	1.67	0.00	2.50
		Control	0.00	0.00	0.83	1.67	0.00	2.50
	% Fresh Weight Reduction	Inoculated	51.92	3.51	40.50	12.22	-10.25	11.11
		Control	0.00	0.00	0.00	0.00	0.00	0.00

00-003C4	% Disease: Week 1	Inoculated	0.00	0.83	6.67	6.67	1.67	0.00
		Control	0.00	0.00	0.00	10.00	0.00	0.83
	% Disease: Week 2	Inoculated	1.67	0.00	2.50	1.67	0.00	0.00
		Control	0.00	0.83	0.00	1.67	5.00	0.00
	% Fresh Weight Reduction	Inoculated	2.45	-2.89	44.94	-8.78	-9.58	-5.22
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-063C2	% Disease: Week 1	Inoculated	6.67	2.50	30.00	15.00	15.00	6.67
		Control	0.00	0.00	0.00	2.50	1.67	1.67
	% Disease: Week 2	Inoculated	2.50	10.83	30.00	15.00	2.50	2.50
		Control	0.00	0.00	0.00	6.67	0.83	0.83
	% Fresh Weight Reduction	Inoculated	51.92	11.64	39.21	3.45	-3.56	3.15
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-098B2	% Disease: Week 1	Inoculated	1.67	2.50	10.83	10.83	2.50	0.00
		Control	0.00	1.67	10.83	2.50	12.50	0.00
	% Disease: Week 2	Inoculated	1.67	2.50	6.67	15.00	2.50	0.00
		Control	0.00	5.83	18.33	15.00	13.33	0.00
	% Fresh Weight Reduction	Inoculated	23.61	35.27	-47.02	-42.30	-2.17	24.78
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-098C2	% Disease: Week 1	Inoculated	70.83	87.50	87.50	87.50	62.50	2.50
		Control	0.00	1.67	10.83	2.50	12.50	0.00
	% Disease: Week 2	Inoculated	30.83	62.50	87.50	79.17	54.17	0.00
		Control	0.00	5.83	18.33	15.00	13.33	0.00
	% Fresh Weight Reduction	Inoculated	90.89	86.49	96.36	89.49	80.72	5.92
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-108C	% Disease: Week 1	Inoculated	0.83	0.00	10.83	2.50	0.83	0.83
		Control	0.00	0.00	0.00	10.00	0.00	0.83
	% Disease: Week 2	Inoculated	2.50	0.00	2.50	2.50	0.83	0.00
		Control	0.00	0.83	0.00	1.67	5.00	0.00
	% Fresh Weight Reduction	Inoculated	37.36	-30.96	31.12	19.93	-1.70	-9.88
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-111B	% Disease: Week 1	Inoculated	1.67	2.50	6.67	10.83	1.67	0.00
		Control	0.00	1.67	10.83	2.50	12.50	0.00
	% Disease: Week 2	Inoculated	2.50	2.50	10.83	15.00	2.50	0.00
		Control	0.00	5.83	18.33	15.00	13.33	0.00
	% Fresh Weight Reduction	Inoculated	7.42	38.81	-37.58	-6.48	-15.29	5.29
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-114E	% Disease: Week 1	Inoculated	0.83	0.83	15.00	2.50	5.83	2.50
		Control	0.00	0.00	0.00	10.00	0.00	0.83
	% Disease: Week 2	Inoculated	6.67	0.00	6.67	2.50	2.50	0.00
		Control	0.00	0.83	0.00	1.67	5.00	0.00
	% Fresh Weight Reduction	Inoculated	33.70	-36.23	48.96	-9.11	-0.51	9.53
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-190A2	% Disease: Week 1	Inoculated	1.67	0.83	6.67	17.50	0.00	15.00
		Control	0.00	0.00	5.83	5.83	0.00	15.00
	% Disease: Week 2	Inoculated	15.00	0.83	2.50	5.83	0.00	2.50
		Control	0.00	0.00	0.83	1.67	0.00	2.50
	% Fresh Weight Reduction	Inoculated	35.51	1.03	-1.48	19.89	-16.18	0.89
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-193C	% Disease: Week 1	Inoculated	6.67	0.00	5.83	10.00	0.00	2.50
		Control	0.00	0.00	6.67	0.00	0.00	1.67
	% Disease: Week 2	Inoculated	6.67	0.00	1.67	17.50	0.00	0.00
		Control	0.00	0.00	2.50	0.00	0.00	0.83

	% Fresh Weight Reduction	Inoculated	31.86	14.06	14.45	31.16	-11.83	14.86
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-215A	% Disease: Week 1	Inoculated	0.00	0.83	1.67	6.67	0.83	1.67
		Control	0.00	1.67	2.50	15.00	0.83	2.50
	% Disease: Week 2	Inoculated	1.67	0.83	1.67	10.83	0.00	0.00
		Control	0.00	1.67	2.50	10.83	5.00	0.00
	% Fresh Weight Reduction	Inoculated	33.85	11.60	2.64	-17.82	6.60	-17.41
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-215B	% Disease: Week 1	Inoculated	45.83	5.83	0.00	0.00	0.00	2.50
		Control	0.00	5.83	0.00	0.00	0.00	2.50
	% Disease: Week 2	Inoculated	22.50	5.83	0.00	0.00	5.00	0.00
		Control	0.00	6.67	0.00	0.00	0.00	0.00
	% Fresh Weight Reduction	Inoculated	59.93	-29.05	-8.83	-25.50	1.57	-4.14
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-246A	% Disease: Week 1	Inoculated	1.67	1.67	2.50	6.67	0.00	2.50
		Control	0.00	0.83	2.50	0.83	0.00	5.83
	% Disease: Week 2	Inoculated	1.67	0.83	2.50	6.67	0.00	2.50
		Control	0.00	5.83	2.50	6.67	0.00	2.50
	% Fresh Weight Reduction	Inoculated	7.28	9.33	-9.12	21.83	21.39	-7.07
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-248A1	% Disease: Week 1	Inoculated	62.50	1.67	0.83	0.83	0.00	6.67
		Control	0.00	5.83	0.00	0.00	0.00	2.50
	% Disease: Week 2	Inoculated	37.50	0.83	0.00	0.83	0.00	0.00
		Control	0.00	6.67	0.00	0.00	0.00	0.00
	% Fresh Weight Reduction	Inoculated	78.16	-40.80	5.03	-4.12	-36.64	-5.73
		Control	0.00	0.00	0.00	0.00	0.00	0.00
00-262B	% Disease: Week 1	Inoculated	0.00	2.50	30.00	10.83	2.50	6.67
		Control	0.00	0.00	0.00	2.50	1.67	1.67
	% Disease: Week 2	Inoculated	0.00	1.67	25.83	15.00	2.50	10.83
		Control	0.00	0.00	0.00	6.67	0.83	0.83
	% Fresh Weight Reduction	Inoculated	1.21	0.37	56.15	8.27	-2.29	-0.45
		Control	0.00	0.00	0.00	0.00	0.00	0.00
01-021A	% Disease: Week 1	Inoculated	1.75	NT	NT	5.75	0.00	32.13
		Control	0.00	NT	NT	0.00	0.00	0.88
	% Disease: Week 2	Inoculated	1.75	NT	NT	4.88	0.00	16.00
		Control	0.00	NT	NT	0.00	0.00	0.00
	% Fresh Weight Reduction	Inoculated	-4.13	NT	NT	35.08	-12.72	44.98
		Control	0.00	NT	NT	0.00	0.00	0.00
01-026B1	% Disease: Week 1	Inoculated	26.75	NT	NT	1.75	0.00	0.88
		Control	0.00	NT	NT	0.88	0.00	0.00
	% Disease: Week 2	Inoculated	21.38	NT	NT	4.88	0.00	0.00
		Control	0.00	NT	NT	9.38	0.00	0.00
	% Fresh Weight Reduction	Inoculated	64.71	NT	NT	32.09	-4.93	7.32
		Control	0.00	NT	NT	0.00	0.00	0.00
01-035B2	% Disease: Week 1	Inoculated	29.88	NT	NT	32.13	0.00	37.50
		Control	0.00	NT	NT	0.00	0.00	0.88
	% Disease: Week 2	Inoculated	18.25	NT	NT	12.88	0.00	37.50
		Control	0.00	NT	NT	0.00	0.00	0.00
	% Fresh Weight Reduction	Inoculated	51.22	NT	NT	51.26	-10.83	35.47
		Control	0.00	NT	NT	0.00	0.00	0.00
01-040	% Disease: Week 1	Inoculated	50.88	NT	NT	0.88	0.00	0.00
		Control	0.00	NT	NT	0.88	0.00	0.00

	% Disease: Week 2	Inoculated	56.25	NT	NT	1.75	0.00	0.00
	% Fresh Weight Reduction	Control	0.00	NT	NT	9.38	0.00	0.00
		Inoculated	75.82	NT	NT	22.21	3.40	13.13
		Control	0.00	NT	NT	0.00	0.00	0.00
01-045A1	% Disease: Week 1	Inoculated	3.50	NT	NT	0.00	0.00	0.00
		Control	0.00	NT	NT	0.00	0.00	0.88
	% Disease: Week 2	Inoculated	12.88	NT	NT	0.00	0.00	0.00
		Control	0.00	NT	NT	0.00	0.00	0.00
	% Fresh Weight Reduction	Inoculated	53.28	NT	NT	-2.21	2.14	3.89
		Control	0.00	NT	NT	0.00	0.00	0.00
01-053A1	% Disease: Week 1	Inoculated	0.00	NT	NT	0.00	0.00	0.00
		Control	0.00	NT	NT	0.88	0.00	0.00
	% Disease: Week 2	Inoculated	0.88	NT	NT	0.00	0.00	0.00
		Control	0.00	NT	NT	9.38	0.00	0.00
	% Fresh Weight Reduction	Inoculated	33.99	NT	NT	-5.31	5.43	-0.06
		Control	0.00	NT	NT	0.00	0.00	0.00
01-054A	% Disease: Week 1	Inoculated	0.00	NT	NT	0.00	0.00	0.00
		Control	0.00	NT	NT	0.00	0.00	0.88
	% Disease: Week 2	Inoculated	0.00	NT	NT	0.00	0.00	0.00
		Control	0.00	NT	NT	0.00	0.00	0.00
	% Fresh Weight Reduction	Inoculated	36.02	NT	NT	9.35	-14.81	-23.56
		Control	0.00	NT	NT	0.00	0.00	0.00
01-060	% Disease: Week 1	Inoculated	6.63	NT	NT	0.88	0.00	0.00
		Control	0.00	NT	NT	0.88	0.00	0.00
	% Disease: Week 2	Inoculated	18.25	NT	NT	0.88	0.00	0.00
		Control	0.00	NT	NT	9.38	0.00	0.00
	% Fresh Weight Reduction	Inoculated	55.72	NT	NT	7.33	-2.57	-5.36
		Control	0.00	NT	NT	0.00	0.00	0.00
01-080A1	% Disease: Week 1	Inoculated	96.50	NT	NT	96.50	96.50	12.88
		Control	0.00	NT	NT	0.88	0.00	0.00
	% Disease: Week 2	Inoculated	96.50	NT	NT	96.50	96.50	16.00
		Control	0.00	NT	NT	9.38	0.00	0.00
	% Fresh Weight Reduction	Inoculated	100.00	NT	NT	95.32	92.77	29.09
		Control	0.00	NT	NT	0.00	0.00	0.00

III. T-tests for expaned host-range studies:

Isolate 00-079E

Crop	Mean A	Mean B	t-value	df	p	Std.Dev A	Std.Dev B
Wheat	10.83	2.50	2.00	4	.116117	7.22	0.00
Barley	2.50‡	0.00	--	4	--	0.00	0.00
Oats	10.83	10.83	0.00	4	1.000000	7.22	7.22
Argentine Canola	10.83	0.00	2.60	4	.060048	7.22	0.00
Polish Canola	45.83*	0.00	5.50	4	.005328	14.43	0.00
Mustard	46.67*	0.00	2.95	4	.042079	27.42	0.00
Lentil	87.50*	10.83	18.40	4	.000051	0.00	7.22

Chickpea	54.17*	0.00	6.50	4	.002890	14.43	0.00
Field Pea	15.00‡	2.50	--	4	--	0.00	0.00
Alfalfa	22.50	2.50	2.67	4	.056000	12.99	0.00
Flax	63.33	0.83	2.58	4	.061026	41.86	1.44
Pineapple Weed	70.83*	0.00	4.25	4	.013158	28.87	0.00
Ox-eye Daisy	6.67	20.83	-0.67	4	.541396	7.22	36.28
Scentless Chamomile	22.50*	0.00	3.00	4	.039942	12.99	0.00

Isolate 00-003B1

Crop	Mean A	Mean B	t-value	df	p	Std.Dev A	Std.Dev B
Wheat	0.83	0.00	1.00	4	.373901	1.44	0.00
Barley	0.83	0.00	1.00	4	.373901	1.44	0.00
Oats	1.67	0.00	2.00	4	.116117	1.44	0.00
Argentine Canola	0.00	0.00	--	4	--	0.00	0.00
Polish Canola	0.00	0.00	--	4	--	0.00	0.00
Mustard	0.00	0.00	--	4	--	0.00	0.00
Lentil	0.83	0.00	1.00	4	.373901	1.44	0.00
Chickpea	0.00	6.67	-1.60	4	.184849	0.00	7.22
Field Pea	0.00	0.00	--	4	--	0.00	0.00
Alfalfa	0.00	0.00	--	4	--	0.00	0.00
Flax	0.00	0.00	--	4	--	0.00	0.00
Pineapple Weed	0.833	0.00	1.00	4	.373901	1.44	0.00
Ox-eye Daisy	0.00	0.00	--	4	--	0.00	0.00
Scentless Chamomile	15.00‡	0.00	--	4	--	0.00	0.00
German Chamomile	6.67	0.00	1.60	4	.184849	7.22	0.00

A = inoculated; B = control; * = statistically significantly different; ‡ = significantly different, unable to analyze due to lack of variability

Isolate 00-002B3

Crop	Mean A	Mean B	t-value	df	p	Std.Dev A	Std.Dev B
Wheat	0.833	0.00	1.00	4	.373901	1.44	0.00
Barley	0.00	0.00	--	4	--	0.00	0.00
Oats	2.50‡	0.00	--	4	--	0.00	0.00
Argentine Canola	5.00	0.00	1.00	4	.373901	8.66	0.00
Polish Canola	0.00	0.00	--	4	--	0.00	0.00
Mustard	0.00	0.00	--	4	--	0.00	0.00
Lentil	0.00	0.00	--	4	--	0.00	0.00
Chickpea	0.00	6.67	-1.60	4	.184849	0.00	7.22
Field Pea	0.00	0.00	--	4	--	0.00	0.00
Alfalfa	0.00	0.00	--	4	--	0.00	0.00
Flax	0.00	0.00	--	4	--	0.00	0.00
Pineapple Weed	2.50‡	0.00	--	4	--	0.00	0.00
Ox-eye Daisy	0.00	0.00	--	4	--	0.00	0.00
Scentless Chamomile	10.83	0.00	2.60	4	.060048	7.22	0.00
German Chamomile	2.50‡	0.00	--	4	--	0.00	0.00

Isolate 00-193C

Crop	Mean A	Mean B	t-value	df	p	Std.Dev A	Std.Dev B
Wheat	2.50	2.50	--	6	--	0.00	0.00
Barley	1.88*	0.00	3.00	6	.024008	1.25	0.00
Oats	5.63	11.88	-1.41	6	.207031	6.25	6.25
Argentine Canola	0.00	1.25	-1.73	6	.133975	0.00	1.44
Polish Canola	0.00	0.00	--	6	--	0.00	0.00
Mustard	0.63	0.00	1.00	6	.355918	1.25	0.00
Lentil	5.63	2.50	1.00	6	.355918	6.25	0.00
Chickpea	5.63	0.63	1.57	6	.167714	6.25	1.25
Field Pea	1.25	0.63	0.65	6	.536963	1.44	1.25
Alfalfa	0.00	0.00	--	6	--	0.00	0.00
Flax	1.25	0.63	0.65	6	.53963	1.44	1.25
Pineapple Weed	0.00	0.00	--	6	--	0.00	0.00
Ox-eye Daisy	0.00	0.00	--	6	--	0.00	0.00
Scentless Chamomile	11.88*	0.00	3.80	6	.008968	6.25	0.00
German Chamomile	0.63	0.00	1.00	6	.355918	1.25	0.00

Isolate 00-215B

Crop	Mean A	Mean B	t-value	df	p	Std.Dev A	Std.Dev B
Wheat	2.50	2.50	--	6	--	0.00	0.00
Barley	2.50‡	0.00	--	6	--	0.00	0.00
Oats	2.50*	11.88	-3.00	6	.024088	0.00	6.25
Argentine Canola	0.00	1.25	-1.73	6	.133975	0.00	1.44
Polish Canola	0.63	0.00	1.00	6	.355918	1.25	0.00
Mustard	0.00	0.00	--	6	--	0.00	0.00
Lentil	1.88	2.50	-1.00	6	.355918	1.25	0.00
Chickpea	0.00	0.00	--	6	--	0.00	0.00
Field Pea	1.25	.63	0.65	6	.536963	1.44	1.25
Alfalfa	0.00	0.00	--	6	--	0.00	0.00
Flax	0.63	0.63	0.00	6	1.00	1.25	1.25
Pineapple Weed	1.25	0.00	1.73	6	.133975	1.44	0.00
Ox-eye Daisy	0.00	0.00	--	6	--	0.00	0.00
Scentless Chamomile	15.00‡	0.00	--	6	--	0.00	0.00
German Chamomile	0.00	0.00	--	6	--	0.00	0.00

Isolate 00-248A1

Crop	Mean A	Mean B	t-value	df	p	Std.Dev A	Std.Dev B
Wheat	2.50	2.50	--	6	--	0.00	0.00
Barley	1.25	1.88	-0.65	6	.536963	1.44	1.25
Oats	2.50	1.88	1.00	6	.355918	0.00	1.25
Argentine Canola	0.00	0.63	-1.00	6	.355918	0.00	1.25
Polish Canola	2.50‡	0.00	--	6	--	0.00	0.00
Mustard	1.88	1.25	0.65	6	.53693	1.25	1.44
Lentil	15.00‡	2.50	--	6	--	0.00	0.00
Chickpea	0.00	0.00	--	6	--	0.00	0.00
Field Pea	0.00	0.00	--	6	--	0.00	0.00
Alfalfa	0.00	0.00	--	6	--	0.00	0.00
Flax	11.88*	0.00	3.80	6	.008968	6.25	0.00
Pineapple Weed	0.00	0.00	--	6	--	0.00	0.00
Ox-eye Daisy	0.00	0.00	--	6	--	0.00	0.00
Scentless Chamomile	20.63*	0.00	3.67	6	.010496	11.25	0.00
German Chamomile	0.00	0.00	--	6	--	0.00	0.00

Isolate 00-190A2

Crop	Mean A	Mean B	t-value	df	p	Std.Dev A	Std.Dev B
Wheat	2.50	2.50	--	6	--	0.00	0.00
Barley	1.88	1.88	0.00	6	1.00	1.25	1.25
Oats	2.50	1.88	1.00	6	.355918	0.00	1.25
Argentine Canola	2.50*	0.63	3.00	6	.024008	0.00	1.25
Polish Canola	7.50	0.00	1.73	6	.133975	8.66	0.00
Mustard	8.13	1.25	1.69	6	.141860	8.00	1.44
Lentil	15.00‡	2.50	--	6	--	0.00	0.00
Chickpea	0.00	0.00	--	6	--	0.00	0.00
Field Pea	0.00	0.00	--	6	--	0.00	0.00
Alfalfa	0.63	0.00	1.00	6	.355918	1.25	0.00
Flax	11.88*	0.00	3.80	6	.008968	6.25	0.00
Pineapple Weed	0.00	0.00	--	6	--	0.00	0.00
Ox-eye Daisy	0.00	0.00	--	6	--	0.00	0.00
Scentless Chamomile	15.00‡	0.00	--	6	--	0.00	0.00
German Chamomile	5.00	0.00	1.48	6	.190116	6.77	0.00

A = inoculated; B = control; * = statistically significantly different; ‡ = significantly different, unable to analyze due to lack of variability